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(54) Title: PEPTIDES USEFUL FOR INDUCING TOLERANCE

Sequence

peptide name	
X	KRDVDLFLTGTPDEYVEQVAQYKALPV
Y	KALPVVLENARILKNCVDAKMTEEDKE
Z	FFAVANGNELLLDLSLTKVNATEPER
A	EEDKENALSLLDKIYTSPL
B	MGEAVQNTVEDLKLNTLGR
C	EEDKENALSLLDKIYT
D	NALSLLDKIYTSPL
E	TEEDKENALSLLDKIYTSPL

(57) Abstract

The present invention is based on the discovery that subcutaneous administration of a peptide comprising at least one T cell epitope of a protein allergen or other protein antigen results in T cell tolerance upon subsequent exposure to the allergen or other antigen. Peptides useful for inducing tolerance in a mammal, such as a human by subcutaneous administration or other routes of administration are within the scope of this invention. Such peptides include peptide X (SEQ ID NO: 7), peptide Y (SEQ ID NO: 8), peptide Z (SEQ ID NO: 9), peptide A (SEQ ID NO: 10), peptide B (SEQ ID NO: 11), peptide C (SEQ ID NO: 12), peptide D (SEQ ID NO: 13), and peptide E (SEQ ID NO: 14), each as shown in the figure. It is preferred that such peptides be administered in soluble form.

PEPTIDES USEFUL FOR INDUCING TOLERANCE

Background of the Invention

T lymphocytes can mediate and regulate both the specific and non-specific effector mechanisms of immune responses. CD4+ T lymphocytes provide help for antibody production and secrete cytokines which modulate the growth of other T cells and the growth and differentiation of other immune cells such as monocytes and granulocytes. Functional and biochemical studies have demonstrated that the generation of cellular immune responses depends upon antigen receptors on T cells that recognize peptide fragments of foreign proteins associated with products of the major histocompatibility complex (MHC) that are expressed on antigen-presenting accessory cells.

Experiments with human and mouse T cell clones have demonstrated that the interaction of the T cell receptor and the peptide/MHC protein complex leads to either activation or inactivation of the cell's ability to respond to subsequent peptide/MHC challenges (Lamb, J.R., et al. (1983) J. of Exp. Med. 157:1434-1447; Zander, E.D., et al. (1983) Nature 303:625-627; and Quill, H. and Swartz, R.H. (1987) J. of Immunol. 138:3704-3712). Whether T cell receptor engagement activates or inhibits the T cell's ability to subsequently respond to the peptide/MHC protein complex may be determined by the presence or absence of costimulatory signals delivered to the T cell by the antigen-presenting cell (Jenkins, M.K., et al. (1990) J. of Immunol. 144:1585-1590; and Jenkins, M.K. and Schwartz, R.H. (1987) J. of Exper. Med. 165:302-319). An example is the signal generated by

CD28 binding of B7 (Jenkins, M.K., _et al._ (1991) _The J. of Immunol._ 147:2461-2466; Linsley, P.S., _et al._ (1990) _PNAS USA_ 5031-5035). Costimulatory events of this type appear to be an obligate requirement for T

05 cell activation even when the T cell receptor is bound to an appropriate MHC protein and peptide. Interestingly, if there is no second signal, then the antigen-specific T cells will become tolerized, or enter into a state of anergy. Additionally, _in vivo_

10 tolerance to antigen challenge has been shown using soluble antigens (Dietrich, F.M. and Weigle, W.O. (1964) _J. of Immunol._ 92:167-172; Mitchinson, N.A. (1964) _Proc. Roy. Soc. B._ 161:275-292) or immunogenic peptides administered in such a way as to preclude

15 the second signal (Gammon, G. and Sercarz, E.E. (1989) _Nature_ 342:183-185; Ria, F., _et al._ (1990) _Nature_ 343:371-383). Peripheral T cell tolerance to endogenous antigens has also been demonstrated in MHC gene transgenic mice _in vivo_ (Burkley, L.C., _et al._

20 (1989) _Nature_ 342:564-566, Reviewed in Miller, J.F.A., _et al._ (1991) _Immunol. Reviews_ 122:103-116 and Hammerling, G.J., _et al._ (1991) _Immunol. Reviews_ 122:47-67).

Recent advances in technology have made it

25 possible to efficiently culture antigen-specific human and mouse T cell lines and clones _in vitro_. In addition, it is now possible to produce large amounts of protein antigens or their fragments using recombinant DNA technology or solid phase peptide

30 synthesis. Thus, in the last few years, several research groups have begun to determine the linear amino acid sequences of antigenic proteins that are recognized by T cells in association with MHC (T cell epitopes). Peptides derived from a variety of

protein antigens, including bacterial and viral
pathogens, autoantigens, allergens and other
experimental antigens such as hen egg lysozyme (HEL),
ovalbumin (OVA) and lambda repressor (cl) have been
05 examined for the ability to stimulate
antigen-specific T cells. A wide spectrum of
peptides has been reported to serve as T cell
epitopes. For example, OVA amino acid residues
324-339 (Shimonkevitz, R. et al., J. Immunol.,
10 133:2167 (1984)), HEL amino acid residues 74-96
(Shastri, N. et al., J. Exp. Med. 164:882-896 (1986);
and lambda repressor (cl) amino acid residues 12-26
(Lai, M.-Z et al., J. Immunol., 139:3973-3980 (1987))
have been demonstrated to stimulate whole
15 protein-primed T cells.

In addition, a peptide derived from Hepatitis B
surface antigen (HBsAg amino acid residues 19-33) has
recently been shown to stimulate T cell responses in
a majority of human subjects who had been immunized
20 with a recombinant hepatitis B vaccine (Schad, V.C.
et al., Seminars in Immunol., 3:217-224 (1991)). A
major mycobacterial antigen 65-kD protein has also
been epitope-mapped (Lamb, J.R. et al., EMBO J.,
6(5):1245-1249 (1987)). T cell epitopes have been
25 identified in the peptides comprised of amino acid
residues 112-132 and 437-459 of the 65-kD protein.
Myelin basic protein (MBP), an autoantigen which
induces experimental autoimmune encephalomyelitis
(EAE) and the presumed autoantigen in multiple
30 sclerosis (MS) has also been epitope-mapped in both
human (Ota, K. et al., Nature, 346:183-187 (1990))
and rodent (Zamvil et al., Nature 324:258-260(1986))
systems. Ota et al. have identified a major T cell
epitope recognized by MS patients, MBP amino acid

residues 84-102. Minor epitopes (MBP amino acid residues 143-168, 61-82, 124-42 and 31-50) recognized by T cells from MS patients were also described. Zamvil *et al.* have shown that MBP amino acid residues

05 1-11 contain the major T cell epitope(s) causing EAE, in susceptible rodent strains.

T cell epitopes present in allergenic proteins have very recently been described (O'Hehir, R. *et al.*, *Ann. Rev. Immunol.*, 9:67-95 (1991)). Several

10 peptides derived from the house dust mite allergen *Der p* I have been shown to be T cell-reactive (Thomas, W.R., *et al.*, In *Epitopes of Atopic Allergens Proceedings of Workshop from XIV Congress of the European Academy of Allergy and Clinical*

15 *Immunology*, Berlin (Sept. 1989) pp. 77-82; O'Hehir, R.E. *Annual Review Immunology* 9:67-95 (1991); Stewart, G.A. *et al.*, In: *Epitopes of Atopic Allergens Proceedings of Workshop from XIV Congress of the European Academy of Allergy and Clinical*

20 *Immunology*, Berlin (Sept. 1989) pp 41-47; and Yessel, H. *et al.*, In: *T Cell Activation in Health and Disease: Discrimination Between Immunity and Tolerance*, Conference 22-26 (Sept. 1990) Trinity College, Oxford U.K.). A T cell-stimulatory peptide

25 derived from the short ragweed allergen *Amb a* I amino acid residues 54-65 has also been reported (Rothbard, J.B. *et al.*, *Cell*, 52: 515-523 (1988). Using a panel of T cell clones derived from a rye grass-allergic individual, Perez *et al.* demonstrated that T cell

30 epitopes are contained within amino acid residues 191-210 of the protein allergen *Lol p* I (Perez, M. *et al.*, *J. Biol. Chem.*, 265(27):16210-16215 (1990)).

Summary of the Invention

Effective intervention in allergic and other immune response mediated diseases requires altering an established or ongoing immune response. The

05 present invention is based on the discovery that subcutaneous administration of a peptide to a mammal decreases the T cell response to subsequent challenge with the same peptide, whether a mammal is naive or preexposed to the protein allergen or other antigen

10 from which the peptide is derived. This invention also relates to peptides useful for tolerization by subcutaneous or other routes of administration. For such tolerization to be clinically useful, it must be effective upon environmental exposure to whole

15 protein allergen or other protein antigen. Thus, subcutaneous adminstration of a peptide comprising at least one T cell epitope of a protein allergen or other protein antigen results in T cell tolerization or anergy upon exposure to the allergen or other

20 antigen. Such peptide-induced T cell tolerance can be used to treat immune mediated diseases such as allergy. The ability to tolerize with subcutaneous injection of a peptide rather than intravenous or intraperitoneal injection provides a more practical

25 and efficient therapeutic approach.

Peptides useful in methods of tolerizing a mammal, such as a human can be derived from protein allergens. These peptides comprise at least one T cell epitope of an allergen and preferably have

30 minimal immunoglobulin E stimulating activity. In addition, for therapeutic purposes, it is preferred that such peptides bind immunoglobulin E to a substantially lesser extent than the protein allergen from which the peptides is derived binds

35 immunoglobulin E. More preferably, peptides derived

Figure 6 is a graphic representation of the dose response necessary to induce T cell tolerance with subcutaneous administration of peptide X in mice.

Figure 7 is a graphic representation of the induction of T cell tolerance in mice by administration of a combination of peptide X and peptide Y subcutaneously followed by challenge with both peptide X and peptide Y. Lymph node cells were isolated, cultured in vitro with a combination of peptide X and peptide Y or with peptide X or peptide Y separately and tested for the presence of IL-2.

Figure 8 is a graphic representation of the response of mice preimmunized with TRFP upon administration of peptide Y.

Figure 9A is a graphic representation of the induction of T cell tolerance in mice primed with TRFP and tolerized with peptide Y. Lymph node cells were isolated, cultured in vitro with peptide Y and tested for the presence of IL-2. Figure 9B is a graphic representation of the induction of IgG tolerance in mice primed with TRFP and tolerized with peptide Y. A sera sample was obtained and assayed for binding to peptide Y in a standard ELISA assay.

Figures 10A and 10B are graphic representations of the induction of T cell tolerance in mice primed with TRFP and tolerized with peptide Y. Lymph node cells were isolated, cultured in vitro with peptide Y and tested for the presence of IL-2 (culture with CTLL-3) or IL-4 (culture with CT4S).

Figure 11 is a graphic representation of the induction of T cell tolerance in mice with recombinant chain 1 of TRFP (peptide Y and peptide X) peptides Fel 3-1, peptide Y, peptide C and peptide D. The mice were challenged with recombinant chain 1

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of TRFP. Lymph node cells were isolated, cultured
with recombinant chain 1 of TRFP and tested for the
presence of IL-2.

Figure 12 is a graphic representation of the
results of direct binding studies of human IgE to
TRFP, peptide X and peptide Y.

Figure 13 is a graphic representation of the
results of histamine release from basophils in
response to TRFP, peptide X and peptide Y.

Figure 14 shows the amino acid sequences of the
following peptides: Fel 33, Fel 34, Fel 35, Fel 36,
Fel 37, Fel 38, Fel 38-1, Fel 39 and Fel 39.1.

Figure 15A, B and C is a graphic representation depicting
the response of T cells from patient #688 primed in
vitro to TRFP and analyzed for response to various
peptides derived from TRFP as measured by Il-2
production and Il-4 production.

Figure 16A, B and C is a graphic representation depicting
the response of T cells from patient #730 primed in
vitro to TRFP and analyzed for response to various
peptides derived from TRFP as measured by Il-2
production and IL-4 production.

Figure 17A, B and C is a graphic representation depicting
the response of T cells from patient #738 primed in
vitro to TRFP and analyzed for response to various
peptides derived from TRFP as measured by Il-2
production and IL-4 production.

Figure 18A, B and C is a graphic representation depicting
the response of T cells from patient #807 primed in
vitro to TRFP and analyzed for response to various
peptides derived from TRFP as measured by Il-2
production and IL-4 production.

Figure 19A, B and C is a graphic representation depicting
the response of T cells from patients #688, #730,

#738, and #807 primed in vitro to TRFP and analyzed for response to various peptides derived from TRFP as measured by Il-2 production and Il-4 production.

Figure 20 is a graph depicting the ratio of Il-4 production to Il-2 production by T cell lines from cat allergic patients, #688, #730, #738, and #807 in response to various peptides derived from TRFP.

Figure 21 is a graph depicting the results of a direct ELISA assay in which various antigens were incubated with human plasma obtained from a cat allergic individual.

Figure 22 is a graph depicting the results of a depletion ELISA assay in which human plasma obtained from a cat allergic individual was preabsorbed on various antigens followed by incubation on fresh antigen coated plates.

Detailed Description of the Invention

The present invention is based on the discovery that subcutaneous administration of a peptide comprising at least one T cell epitope of a protein allergen or other protein antigen results in T cell tolerance upon subsequent exposure to the allergen or other antigen. Peptides useful for inducing tolerance in a mammal, such as a human by subcutaneous administration or other routes of administration are within the scope of this invention. Such peptides include peptide X (SEQ ID NO: 7), peptide Y (SEQ ID NO: 8), peptide Z (SEQ ID NO: 9), peptide A (SEQ ID NO: 10), peptide B (SEQ ID NO: 11), peptide C (SEQ ID NO: 12), peptide D (SEQ ID NO: 13), and peptide E (SEQ ID NO: 14), each as shown in Figure 3. It is preferred that such peptides be administered in soluble form.

        In general, peptides useful in methods of
tolerization have human T cell stimulating activity
as determined by standard T cell biology techniques
and, thus, comprise at least one T cell epitope. If
05 desired, precise T cell epitopes can be determined
by, for example, fine mapping techniques. This
technique involves modifying a peptide comprising at
least one T cell epitope as defined by standard T
cell biology techniques by addition or deletion of
10 amino acid residues at either the amino or carboxy
terminus of the peptide. Following modification, the
peptide is tested to determine a change in T cell
reactivity. If two or more peptides derived from a
protein antigen share an area of overlap and are both
15 found to have human T cell stimulating activity (as
determined by standard T cell biology techniques)
additional peptides can be produced comprising all or
a portion of such peptides and these additional
peptides can be tested by the above fine mapping
20 procedure. As a result of fine mapping, a set of
human T cell epitopes for a protein allergen or other
antigen comprising amino acid residues essential to T
cell recognition can be produced.
        In the situation where the T cell epitopes of a
25 protein allergen or other protein antigen are unknown
or ill-defined (e.g., some or all of the peptide
regions of the protein antigen which have human T
cell stimulating activity have not been defined by
standard T cell biology techniques or the precise
30 human T cell epitopes of the protein antigen have not
been defined by fine mapping techniques) a peptide
useful in methods of subcutaneous tolerization may be
obtained by reviewing the known protein structure of
an allergen or other antigen and theoretically

dividing the allergen or antigen into at least two
peptide regions of desired lengths. For example, the
protein sequence of the allergen or other antigen can
be systematically divided into at least two
05 non-overlapping peptide regions of desired lengths,
or at least two overlapping peptide regions of
desired lengths. This division into peptide regions
can be arbitrary, can be made according to an
algorithm, or can be wholly or partially based on
10 regions of the protein antigen known to comprise at
least one T cell epitope.

When just a few of the peptide regions of the
protein allergen or other protein antigen comprising
at least one T cell epitope are known or when all the
15 regions of the protein allergen or other protein
antigen which have human T cell stimulating activity
are unknown, preferably, at least 50% of the entire
protein sequence of the protein allergen or other
protein antigen and more preferably, the entire
20 protein sequence of the protein allergen or other
protein antigen is divided into one or more
peptides. The peptide or peptides can then be
produced recombinantly or synthetically and the
ability of the peptide(s) to stimulate human T cells
25 can be determined. A peptide derived from a protein
allergen can be tested to determine whether the
peptide binds immunoglobulin E specific for the
allergen and result in the release of mediators
(e.g., histamine) from mast cells or basophils.
30 Those peptide(s) found to bind immunoglobulin E and
cause the release of mediators from mast cells or
basophils in greater than approximately 10-15% of the
allergic sera tested are preferably not used in
methods of the invention.

Constructing peptides derived from phospholipase
A$_2$, a major allergen from honeybee venom, can be used
as an illustrative example when the protein structure
of a protein antigen is known, but the T cell
05 epitopes are unknown or ill-defined. Phospholipase
A$_2$ is composed of 134 amino acids as defined by cDNA
cloning (Kuchler, K. et al. _Eur. J. Biochem._
184:249-254). This amino acid sequence can be
divided into regions, each preferably containing 20
10 to 35 amino acid residues, each region preferably
overlapping another region by about 10 amino acids.
The entire protein sequence of the protein may be
divided into regions, however, the total sequence
used to determine those peptides useful for
15 subcutaneous administration to an individual can be
substantially less than the entire protein sequence.
To maximize the potential of including T cell
epitopes in the constructed peptide(s), areas of
overlap and length of each region can be designed to
20 maintain the presence of T cell epitopes predicted
using algorithms (see e.g., Rothbard, J. and Taylor,
W.R. _EMBO J._ 7:93-100 (1988); Berzofsky, J.A. _Philos_
Trans R. Soc. Lond. 323:535-544 (1989)). Preferably,
human T cell epitopes within a protein allergen can
25 be predicted using known HLA class II binding
specific amino acid residues. A similar procedure
can be utilized to construct and select peptides
derived from an autoantigen with a known protein
structure, but with undefined T cell epitopes such as
30 glutamic acid decarboxylase (e.g., Samama, J.P., and
Mallet, J. _Journal of Neurochemistry_ 54:703-705
(1990)), insulin (_Joslin's Diabetes Mellitus_, 12th
Edition, Eds. A. Marble et al., Lea & Febiger,
Philadelphia, p. 67 (1985)), etc.

Peptides derived from an allergen or other antigen are tested to determine T cell stimulating activity (i.e., proliferation, lymphokine secretion and/or induction of T cell anergy/ tolerization) and
05 thus comprise at least one T cell epitope. For example, human T cell stimulating activity can be tested by culturing T cells obtained from an individual sensitive to a protein allergen or protein antigen (i.e., an individual who has an immune
10 response to the protein allergen or protein antigen) with a peptide derived from the protein allergen or antigen and determining the presence or absence of proliferation by the T cells in response to the peptide as measured by, for example, uptake of
15 tritiated thymidine. Stimulation indices for responses by T cells to peptides useful in methods of the invention can be calculated as the maximum CPM in response to the peptide divided by the medium control CPM. For example, a peptide derived from a protein
20 allergen may have a stimulation index of about 2.0. A stimulation index of at least 2.0 is generally considered positive for purposes of defining peptides useful as immunotherapeutic agents. Preferred peptides have a stimulation index of at least 2.5,
25 more preferably at least 3.5, and most preferably at least 5.0.

In addition, preferred peptides derived from protein allergens do not bind immunoglobulin E (IgE) or bind IgE to a substantially lesser extent than the
30 protein allergen(s) from which the peptide is derived binds IgE. The major complications of standard immunotherapy are systemic responses such as anaphylaxis. Immunoglobulin E is a mediator of anaphylactic reactions which result from the binding

and cross-linking of antigen to IgE on mast cells or
basophils and the release of mediators (e.g.,
histamine, serotonin, eosinophil chemotactic
factors). Thus, anaphylaxis could be avoided by the
05 use of a peptide which does not bind IgE, or if the
peptide binds IgE, such binding does not result in
the release of mediators (e.g., histamine, etc.) from
mast cells or basophils. In addition, peptides which
have minimal IgE stimulating activity are
10 particularly desirable for therapeutic
effectiveness. Minimal IgE stimulating activity
refers to IgE production that is less than the amount
of IgE production and/or IL-4 production stimulated
by the whole protein allergen.
15   Peptides useful in methods of the invention as
well as the preferred peptides derived from TRFP
(e.g., peptide X, peptide Y, etc.) can be produced by
recombinant DNA techniques in a host cell transformed
with a nucleic acid vector directing expression of a
20 nucleotide sequence coding for such peptide, or by
chemical synthesis, or in certain limited situations
by chemical cleavage of a protein allergen or other
protein antigen. When produced by recombinant
techniques, host cells transformed with nucleic acid
25 vectors directing expression of a nucleotide sequence
coding for a peptide are cultured in a medium
suitable for the cells. The peptides may be secreted
and harvested from a mixture of cells and cell
culture medium. Alternatively, the peptide may be
30 retained cytoplasmically and the cells harvested,
lysed and the peptide isolated and purified.
Peptides can be isolated using techniques known in
the art for purifying peptides or proteins including
ion-exchange chromatography, gel filtration

chromatography, ultrafiltration, electrophoresis, and immunoaffinity purification with antibodies specific for the peptide, the protein allergen or other antigen from which the peptide is derived, or a
05 portion thereof. Any of the peptides described herein are isolated such that the peptide is substantially free of cellular material or culture medium when produced by recombinant DNA techniques, or substantially free of chemical precursors or other
10 chemicals when synthesized chemically, or obtained by chemical cleavage of a protein allergen or other protein antigen.

According to one aspect of the invention, peptides derived from a protein allergen or other
15 antigen, are administered, preferably subcutaneously to a mammal (such as a human) sensitive to the protein allergen or antigen in a form which results in a decrease in the T cell response of the mammal upon subsequent exposure to the allergen or other
20 antigen. As used herein, a decrease or modification of the T cell response of a mammal sensitive to a protein allergen or other antigen can be defined as non-responsiveness or diminution in symptoms to the allergen or other antigen in the mammal, as
25 determined by standard clinical procedures (see e.g., Varney et al., British Medical Journal 302: 265-269 (1990)), including diminution in allergen induced asthmatic symptoms. As referred to herein, a diminution in symptoms to an allergen includes any
30 reduction in the allergic response of a mammal, such as a human, to the allergen following a treatment regimen with a peptide as described herein. This diminution in symptoms may be determined subjectively

protein antigens, including bacterial and viral
pathogens, autoantigens, allergens and other
experimental antigens such as hen egg lysozyme (HEL),
ovalbumin (OVA) and lambda repressor (cl) have been
05 examined for the ability to stimulate
antigen-specific T cells. A wide spectrum of
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When just a few of the peptide regions of the
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at least one T cell epitope are known or when all the
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-13-

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10 response to the protein allergen or protein antigen) with a peptide derived from the protein allergen or antigen and determining the presence or absence of proliferation by the T cells in response to the peptide as measured by, for example, uptake of

15 tritiated thymidine. Stimulation indices for responses by T cells to peptides useful in methods of the invention can be calculated as the maximum CPM in response to the peptide divided by the medium control CPM. For example, a peptide derived from a protein

20 allergen may have a stimulation index of about 2.0. A stimulation index of at least 2.0 is generally considered positive for purposes of defining peptides useful as immunotherapeutic agents. Preferred peptides have a stimulation index of at least 2.5,

25 more preferably at least 3.5, and most preferably at least 5.0.

In addition, preferred peptides derived from protein allergens do not bind immunoglobulin E (IgE) or bind IgE to a substantially lesser extent than the

30 protein allergen(s) from which the peptide is derived binds IgE. The major complications of standard immunotherapy are systemic responses such as anaphylaxis. Immunoglobulin E is a mediator of anaphylactic reactions which result from the binding

and cross-linking of antigen to IgE on mast cells or
basophils and the release of mediators (e.g.,
histamine, serotonin, eosinophil chemotactic
factors). Thus, anaphylaxis could be avoided by the
05 use of a peptide which does not bind IgE, or if the
peptide binds IgE, such binding does not result in
the release of mediators (e.g., histamine, etc.) from
mast cells or basophils. In addition, peptides which
have minimal IgE stimulating activity are
10 particularly desirable for therapeutic
effectiveness. Minimal IgE stimulating activity
refers to IgE production that is less than the amount
of IgE production and/or IL-4 production stimulated
by the whole protein allergen.
15   Peptides useful in methods of the invention as
well as the preferred peptides derived from TRFP
(e.g., peptide X, peptide Y, etc.) can be produced by
recombinant DNA techniques in a host cell transformed
with a nucleic acid vector directing expression of a
20 nucleotide sequence coding for such peptide, or by
chemical synthesis, or in certain limited situations
by chemical cleavage of a protein allergen or other
protein antigen. When produced by recombinant
techniques, host cells transformed with nucleic acid
25 vectors directing expression of a nucleotide sequence
coding for a peptide are cultured in a medium
suitable for the cells. The peptides may be secreted
and harvested from a mixture of cells and cell
culture medium. Alternatively, the peptide may be
30 retained cytoplasmically and the cells harvested,
lysed and the peptide isolated and purified.
Peptides can be isolated using techniques known in
the art for purifying peptides or proteins including
ion-exchange chromatography, gel filtration

chromatography, ultrafiltration, electrophoresis, and immunoaffinity purification with antibodies specific for the peptide, the protein allergen or other antigen from which the peptide is derived, or a
05 portion thereof. Any of the peptides described herein are isolated such that the peptide is substantially free of cellular material or culture medium when produced by recombinant DNA techniques, or substantially free of chemical precursors or other
10 chemicals when synthesized chemically, or obtained by chemical cleavage of a protein allergen or other protein antigen.

According to one aspect of the invention, peptides derived from a protein allergen or other antigen, are administered, preferably subcutaneously
15 to a mammal (such as a human) sensitive to the protein allergen or antigen in a form which results in a decrease in the T cell response of the mammal upon subsequent exposure to the allergen or other
20 antigen. As used herein, a decrease or modification of the T cell response of a mammal sensitive to a protein allergen or other antigen can be defined as non-responsiveness or diminution in symptoms to the allergen or other antigen in the mammal, as
25 determined by standard clinical procedures (see e.g., Varney et al., British Medical Journal 302: 265-269 (1990)), including diminution in allergen induced asthmatic symptoms. As referred to herein, a diminution in symptoms to an allergen includes any
30 reduction in the allergic response of a mammal, such as a human, to the allergen following a treatment regimen with a peptide as described herein. This diminution in symptoms may be determined subjectively

in a human (e.g., the patient feels more comfortable
upon exposure to the allergen), or clinically, such
as with a standard skin test.

As a result of the work described herein,
05 peptides derived from protein allergens or other
protein antigens and having T cell stimulating
activity (i.e., having at least one T cell epitope)
have been produced, for example, the preferred
peptides for use in treating sensitivity to <u>Felis</u>
10 <u>domesticus</u> and derived from TRFP (i.e., peptide X,
peptide Y, peptide Z, peptide A, peptide B, peptide
C, peptide D, peptide E). T cell epitopes are
believed to be involved in initiation and
perpetuation of the immune response to a protein
15 allergen or other protein antigen which is
responsible respectively for the clinical symptoms of
allergy or other diseases. These T cell epitopes are
thought to trigger early events at the level of the T
helper cell by binding to an appropriate HLA molecule
20 on the surface of an antigen presenting cell and
stimulating the relevant T cell subpopulation. These
events lead to T cell proliferation, lymphokine
secretion, local inflammatory reactions, recruitment
of additional immune cells to the site, and
25 activation of the B-cell cascade leading to
production of antibodies. One isotype of these
antibodies, IgE, is fundamentally important to the
development of allergic symptoms and its production
is influenced early in the cascade of events, at the
30 level of the T helper cell, by the nature of the
lymphokines secreted. A T cell epitope is the basic
element or smallest unit of recognition by a T cell
receptor, where the epitope comprises amino acids
essential to receptor recognition and may be

contiguous and/or non-contiguous in the amino acid sequence of the protein. A T cell epitope, as used herein has a stimulation index of at least 2.0, more preferably at least 2.5, even more preferably at

05 least 3.5 and most preferably at least 5.0.

According to the present method, subcutaneous administration of a peptide as described herein to a mammal, such as a human, will tolerize or anergize appropriate T cell subpopulations such that they

10 become unresponsive to the protein allergen or other antigen and do not participate in stimulating an immune response upon such exposure (see Exemplification A). In addition, administration of such a peptide may modify the lymphokine secretion

15 profile as compared with exposure to the naturally-occurring protein allergen or portion thereof (e.g., result in a decrease of IL-4 and/or an increase in IL-2). Furthermore, exposure to the peptide may influence T cell subpopulations which

20 normally participate in the response to the allergen such that these T cells are drawn away from the site(s) of normal exposure to the allergen (e.g., nasal mucosa, skin, and lung) towards the site(s) of therapeutic administration of the peptide. This

25 redistribution of T cell subpopulations may ameliorate or reduce the ability of an individual's immune system to stimulate the usual immune response at the site of normal exposure to the allergen, resulting in a diminution in allergic symptoms.

30 Peptides useful for tolerization can comprise as many amino acid residues as desired and preferably comprise at least about 7, more preferably at least about 15, even more preferably at least about 20 and most preferably at least about 25 amino acid residues

of a protein allergen or other protein antigen. A
peptide length of about 20-40 amino acid residues is
preferred as increases in length of a peptide may
result in difficulty in peptide synthesis as well as
05 retention of an undesirable property (e.g.,
immunoglobulin binding or enzymatic activity) due to
maintenance of conformational similarity between the
peptide and the protein allergen or other protein
antigen from which it is derived. If desired, the
10 amino acid sequences of one or more peptides can be
produced and joined by a linker to increase
sensitivity to processing by antigen-presenting
cells. Such linker can be any non-epitope amino acid
sequence or other appropriate linking or joining
15 agent.

When peptides as used to tolerize an individual
sensitive to a protein allergen by subcutaneous
administration, the peptide is preferably derived
from a protein allergen of the following genus: the
20 genus Dermatophagoides; the genus Felis; the genus
Ambrosia; the genus Lolium; the genus Cryptomeria;
the genus Alternaria; the genus Alder; the genus
Betula; the genus Quercus; the genus Olea; the genus
Artemisia; the genus Plantago; the genus Parietaria;
25 the genus Canis; the genus Blattella, the genus Apis;
and the genus Periplaneta. For example, peptides
useful for subcutaneous tolerization can be derived
from known or unkown protein allergens including
those of the following species: Dermatophagoides
30 pteronyssinus (e.g., Der p I, Der p II);
Dermatophagoides farinae (e.g., Der f I, Der f II);
Ambrosia artemisiifolia (e.g., Amb a I.1, Amb a I.2,
Amb a I.3, Amb a I.4, Amb a II); Cryptomeria japonis
(e.g., Cry j I, Cry j II); Lolium perenne (e.g. Lol p

I, Lol p IX); Felis domesticus (e.g., Fel d I); and
Canis familiaris (e.g., Can f I, Can f II). Peptides
comprising at least one epitope of protein allergens
have been described in the following applications,
05 the contents of which are incorporated herein by
reference: U.S.S.N. 866,679 entitled "T Cell
Epitopes of the Major Allergens from Ambrosia
Artemisiifolia" filed April 9, 1992; and U.S.S.N.
963,381 entitled "T Cell Epitopes of the Major
10 Allergens of Dermatophagoides (House Dust Mite) filed
October 16, 1992.

Particularly preferred peptides for treating
sensitivity to Felis domesticus are derived from the
genus Felis and include the following: peptide X
15 (SEQ ID NO: 7); peptide Y (SEQ ID NO: 8); peptide Z
(SEQ ID NO: 9); peptide A (SEQ ID NO: 10); peptide B
(SEQ ID NO: 11), peptide C (SEQ ID NO: 12); peptide D
(SEQ ID NO: 13); and peptide E (SEQ ID NO: 14), of
TRFP, the amino acid sequences of each peptide as
20 shown in Fig. 3, and modifications thereof. These
preferred peptides from TRFP can be produced
recombinantly or by chemical synthesis as herein
described based on the nucleic acid and amino acid
sequences of TRFP shown in Figs. 1 and 2 (SEQ ID NO:
25 1-6). Such peptides can be administered in the form
of a therapeutic composition to treat sensitivity to
Felis domesticus. Thus, one or more of peptides X,
Y, Z, A, B, C, D and E can be combined in a single
composition or one or more separate compositions for
30 simultaneous or sequential administration to an
individual to be treated for sensitivity to Felis
domesticus. Such compositions can be adminstered
subcutaneously or otherwise, including, but not
limited to intravenously and intraperitoneally in a

form suitable for tolerization. Preferably, the
peptide is administered in soluble form.

   Additional peptides useful for subcutaneous
tolerization can be derived from protein antigens
05 other than protein allergens where modification of an
antigen specific immune response is desired. For
example, a known autoantigen involved in the
pathogenesis of an autoimmune disease can be examined
and peptides having human T cell stimulating activity
10 or peptides having known T cell epitopes can be
identified. One or more of such peptides can be
administered subcutaneously to a mammal, such as a
human, afflicted with an autoimmune disease or at
risk of developing an autoimmune disease to decrease
15 the antibody response to the autoantigen, to
interfere with efficacy and/or decrease immune
complex related side effects. In order to preserve
the T cell reactivity of the autoantigen, peptides of
the autoantigen having human T cell stimulating
20 activity can be defined by standard T cell biology
techniques, or if desired, precise T cell epitopes
can be defined by above-described fine mapping
techniques.

   Peptides which stimulate T cells and do not have
25 undesired properties associated with the autoantigen
(e.g., do not bind autoantibodies in a substantial
percentage of individuals sensitive to the
autoantigen) are selected for use as
immunotherapeutics to tolerize an individual to the
30 autoantigen. In the form of a therapeutic
composition, the peptide would be delivered
subcutaneously in a physiologically acceptable
vehicle in the absence of adjuvant to allow the
peptide to induce antigen specific tolerance to the

autoantigen from which the peptide is derived and
regulate any potentially damaging immune response.
Among autoantigens useful in producing peptides are
insulin, glutamic acid decarboxylase (64K), PM-1 (a
05   69KD autoantigen) and carboxypeptidase in diabetes;
myelin basic protein in multiple sclerosis; rh factor
in erythroblastosis fetalis; acetylcholine receptors
in myasthenia gravis; thyroid receptors in Graves'
Disease; basement membrane proteins in Good Pasture's
10   syndrome; and thyroid proteins in thyroiditis. For
example, known regions of myelin basic protein (MBP)
having human T cell stimulating activity include a
region comprising all or a portion of amino acid
residues 84-106 (preferably amino acid residues
15   84-102 and more preferably amino acid residues
89-101) of human MBP and a region comprising all or a
portion of amino acid residues 140-172 (preferably
amino acid residues 143-168 of human MBP). One or
more peptides comprising each of these regions could
20   be produced and administered subcutaneously to treat
multiple sclerosis in an individual.

        Peptides useful in methods of treating
sensitivity in a mammal, such as a human, to a
protein allergen (including the preferred peptides X,
25   Y, Z, etc. derived from TRFP) or other protein
antigen are in the form of a therapeutic
composition. Such compositions include a peptide and
a pharmaceutically acceptable carrier or diluent.
Administration of the therapeutic compositions of the
30   present invention to desensitize or tolerize an
individual to a protein allergen or other protein
antigen can be carried out using procedures, at
dosages and for periods of time effective to reduce
sensitivity (i.e., reduce the allergic response) of

the individual to a protein allergen or other protein antigen. Effective amounts of the therapeutic compositions will vary according to factors such as the degree of sensitivity of the individual to to the

05 allergen or other antigen, the age, sex, and weight of the individual, and the ability of the peptide(s) to elicit an antigenic response in the individual. Dosage regima may be adjusted to provide the optimum therapeutic response. For example, several divided

10 doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

Peptides derived from TRFP used in treating sensitivity to _Felis domesticus_ (e.g., peptide X,

15 peptide Y, etc.) may be administered in a convenient manner such as by injection (subcutaneous, intravenous, etc.), oral administration, inhalation, transdermal application, or rectal administration. Depending on the route of administration, the active

20 compound may be coated with in a material to protect the compound from the action of enzymes, acids and other natural conditions which may inactivate the compound.

To administer a peptide or peptides of the

25 invention by other than parenteral administration, it may be necessary to coat the peptide with, or co-administer the protein or peptide with, a material to prevent its inactivation. For example, the peptides may be administered to an individual in an

30 appropriate diluent or adjuvant, co-administered with enzyme inhibitors or in an appropriate carrier such as liposomes. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Adjuvant is used in its broadest sense and includes

any immune stimulating compound such as interferon.
Adjuvants contemplated herein include resorcinols,
non-ionic surfactants such as polyoxyethylene oleyl
ether and n-hexadecyl polyethylene ether. Enzyme
05 inhibitors include pancreatic trypsin inhibitor,
diisopropylfluorophosphate (DEP) and trasylol.
Liposomes include water-in-oil-in-water CGF emulsions
as well as conventional liposomes (Strejan et al.,
(1984) J. Neuroimmunol. 7:27). For purposes of
10 inducing T-cell anergy, the therapeutic composition
is preferably administered in non-immunogenic form,
e.g., one that does not contain adjuvant.

The active compound may also be administered
parenterally or intraperitoneally. Dispersions can
15 also be prepared in glycerol, liquid polyethylene
glycols, and mixtures thereof and in oils. Under
ordinary conditions of storage and use, these
preparations may contain a preservative to prevent
the growth of microorganisms.
20 Pharmaceutical compositions suitable for
injectable use include sterile aqueous solutions
(where water soluble) or dispersions and sterile
powders for the extemporaneous preparation of sterile
injectable solutions or dispersion. In all cases,
25 the composition must be sterile and must be fluid to
the extent that easy syringability exists. It must
be stable under the conditions of manufacture and
storage and must be preserved against the
contaminating action of microorganisms such as
30 bacteria and fungi. The carrier can be a solvent or
dispersion medium containing, for example, water,
ethanol, polyol (for example, glycerol, propylene
glycol, and liquid polyetheylene gloycol, and the
like), suitable mixtures thereof, and vegetable

oils. The proper fluidity can be maintained, for
example, by the use of a coating such as licithin, by
the maintenance of the required particle size in the
case of dispersion and by the use of surfactants.
05 Prevention of the action of microorganisms can be
achieved by various antibacterial and antifungal
agents, for example, parabens, chlorobutanol, phenol,
asorbic acid, thimerosal, and the like. In many
cases, it will be preferable to include isotonic
10 agents, for example, sugars, polyalcohols such as
manitol, sorbitol, sodium chloride in the
composition. Prolonged absorption of the injectable
compositions can be brought about by including in the
composition an agent which delays absorption, for
15 example, aluminum monostearate and gelatin.
      Sterile injectable solutions can be prepared by
incorporating active compound in the required amount
in an appropriate solvent with one or a combination
of ingredients enumerated above, as required,
20 followed by filtered sterilization. Generally,
dispersions are prepared by incorporating the active
compound into a sterile vehicle which contains a
basic dispersion medium and the required other
ingredients from those enumerated above. In the case
25 of sterile powders for the preparation of sterile
injectable solutions, the preferred methods of
preparation are vacuum drying and freeze-drying which
yields a powder of the active ingredient (i.e., a
peptide of the invention) plus any additional desired
30 ingredient from a previously sterile-filtered
solution thereof.
      When a preferred peptide or peptides derived
from TRFP, as herein described is suitably protected,
as described above, the peptide may be orally

proteolytic degradation in vivo). A modified peptide
can be produced in which the amino acid sequence has
been altered, such as by amino acid substitution,
deletion, or addition, to modify immunogenicity
05 and/or reduce allergenicity, or to which a component
has been added for the same purpose. For example,
the amino acid residues essential to T cell epitope
function can be determined using known techniques
(e.g., substitution of each residue and determination
10 of presence or absence of T cell reactivity). Those
residues shown to be essential can be modified (e.g.,
replaced by another amino acid whose presence is
shown to enhance T cell reactivity), as can those
which are not required for T cell reactivity (e.g.,
15 by being replaced by another amino acid whose
incorporation enhances T cell reactivity but does not
diminish binding to relevant MHC). Another example
of a modification of peptides is substitution of
cysteine residues preferably with alanine, or
20 glutamic acid, or alternatively with serine or
threonine to minimize dimerization via disulfide
linkages.
        In order to enhance stability and/or reactivity,
peptides can also be modified to incorporate one or
25 more polymorphisms in the amino acid sequence of a
protein allergen resulting from natural allelic
variation. Additionally, D-amino acids, non-natural
amino acids or non-amino acid analogues can be
substituted or added to produce a modified peptide
30 within the scope of this invention. Furthermore,
peptides can be modified using the polyethylene
glycol (PEG) method of A. Sehon and co-workers (Wie
et al. supra) to produce a peptide conjugated with
PEG. Modifications of peptides can also include

reduction/alkylation (Tarr in: Methods of Protein Microcharacterization, J.E. Silver ed. Humana Press, Clifton, NJ, pp 155-194 (1986)); acylation (Tarr, supra); esterification (Tarr, supra); chemical

05 coupling to an appropriate carrier (Mishell and Shiigi, eds, Selected Methods in Cellular Immunology, WH Freeman, San Francisco, CA (1980); U.S. Patent 4,939,239); or mild formalin treatment (Marsh International Archives of Allergy and Applied

10 Immunology 41: 199-215 (1971)).

To facilitate purification and potentially increase solubility of peptides, it is possible to add reporter group(s) to the peptide backbone. For example, poly-histidine can be added to a peptide to

15 purify the peptide on immobilized metal ion affinity chromatography (Hochuli, E. et al., Bio/Technology, 6:1321-1235 (1988)). In addition, specific endoprotease cleavage sites can be introduced, if desired, between a reporter group and amino acid

20 sequences of a peptide to facilitate isolation of peptides free of irrelevant sequences. In order to successfully desensitize an individual to a protein antigen, it may be necessary to increase the solubility of a peptide by adding functional groups

25 to the peptide or by not including hydrophobic T cell epitopes or regions containing hydrophobic epitopes in the peptide.

To potentially aid proper antigen processing of T cell epitopes within a peptide, canonical protease

30 sensitive sites can be recombinantly or synthetically engineered between regions, each comprising at least one T cell epitope. For example, charged amino acid pairs, such as KK or RR, can be introduced between regions within a peptide during recombinant

construction of the peptide. The resulting peptide can be rendered sensitive to cathepsin and/or other trypsin-like enzymes cleavage to generate portions of the peptide containing one or more T cell epitopes.

05 In addition, such charged amino acid residues can result in an increase in solubility of a peptide.

Site-directed mutagenesis of DNA encoding a peptide can be used to modify the structure of the peptide. Such methods may involve PCR (Ho et al.,

10 Gene 77:51-59 (1989)) or total synthesis of mutated genes (Hostomsky, Z., et al., Biochem. Biophys. Res. Comm. 161:1056-1063 (1989)). To enhance bacterial expression, the aforementioned methods can be used in conjunction with other procedures to change the

15 eucaryotic codons in DNA constructs encoding peptides to ones preferentially used in E. coli.

Another aspect of the invention provides peptides shown to have T cell stimulating activity and thus comprising at least one T cell epitope which

20 can be administered to an individual in the form of a therapeutic composition to reduce the individual's response to an allergen of Felis domesticus. Such a therapeutic composition can comprise one or more of such peptides. A preferred composition comprises at

25 least one peptide selected from the group consisting of peptide X (amino acid residues 7-30 from chain 1 of TRFP; also referred to as Fel 8-3 and shown in Figure 3); peptide Y (amino acid residues 29-55 from chain 1 of TRFP; also referred to as Fel 30-4 and

30 shown in Figure 3); peptide Z (amino acid residues 14-39 from chain 2 of TRFP; also referred to as Fel 31-2 and shown in Figure 3); peptide A (amino acid residues 51-69 from chain 1 of TRFP and shown in Figure 3); peptide B (amino acid residues 74-92 from

chain 2 of TRFP; also referred to as Fel 29 and shown in Figure 3); peptide C (amino acid residues 51-66 from chain 1 of TRFP, also referred to as Fel 23 and shown in Figure 3); peptide D (amino acid residues
05   56-69 from chain 1 of TRFP; a modified form of Fel 21 and shown in Figure 3); and peptide E (amino acid residues 50-69 from claim 1 of TRFP and shown in Figure 3).

As described in detail in Exemplification A, T
10   cell tolerance has been induced in mice by subcutaneous or intravenous administration of peptide X or peptide Y separately or in combination. The tolerization of the mice was evidenced by decreases in peptide specific IL-2 production, IL-4 production,
15   and antibody production. Decreases in the T cell activities that are expected to be associated with IgE production, T cell help for B cells and IL-4 production have been shown. In addition, T cells specific for recombinant chain 1 of TRFP were
20   tolerized by administering peptide X and peptide Y.

In another set of experiments, T cell tolerance was induced to myelin basic protein (MBP) in mice by (1) administering one or a combination of two immunodominant T cell determinants from MBP (i.e.,
25   a.a. residues 35-47 and Ac 1-11) prior to immunization with MBP and (2) administering one or a combination of the immunodominant T cell determinants following induction of Experimental Allergic Encephalomelitis (EAE) in the mice. Tolerance
30   induction prior to challenge with MBP blocked the development of severe disease following MPB immunization. "Tolerizing" mice once disease had begun, 10 days following immunization with MBP, blocked disease progession and decreased severity of
35   ongoing disease.

The invention further encompasses at least one
therapeutic composition useful in treating a disease
which involves an immune response to protein antigen
(e.g., an allergen, an autoantigen, etc.) comprising
05   at least one peptide having a sufficient percentage
of the T-cell epitopes of the protein antigen such
that in a substantial percentage of a population of
individuals sensitive to the protein antigen, the
response of such individuals to the protein antigen
10   is substantially diminished, with the provisio that
the at least one peptide does not comprise the entire
protein antigen.

This invention is further illustrated by the
following non-limiting examples.

15   EXEMPLIFICATION A

Peripheral T Cell Tolerance Induced in Naive & Primed
Mice By Subcutaneous Injection of Peptides From the
Human T Cell Reactive Feline Protein (TRFP)

In each of the following experiments the protein
20   and peptides were produced as follows. An
overlapping set of peptides derived from the sequence
of Fel d I chain 1 (referred to herein as Human T
Cell Reactive Feline Protein (TRFP) chain 1)
(Morgenstern, J.P. et al., Proc. Natl. Acad. Sci.
25   (1991) 88:9690-9694)) was synthesized using
conventional Merrifield solid phase t-Boc or Fmoc
chemistry. The peptides were purified by reverse
phase HPLC and examined by amino acid analysis. The
lyophilized peptides were resuspended in PBS and
30   sterilized by gamma irradiation (10,000 rads) or by
passage through a 0.2 μ filter.

Native TRFP protein was purified from an extract of house dust as described by Chapman et al. (J. Immunol. (1988) 140:812-818)). Briefly, house dust (from vacuum containers used in homes with multiple
05 cats) was extracted with PBS, then lyophilized and redissolved in water. The extract was applied to a column coupled with anti-TRFP monoclonal antibody (hybridomas 6F9 and 1G4 were both provided M. Chapman). The TRFP was eluted from the column with
10 100 mM glycine pH 2.5 and was neutralized. Recombinant TRFP chain 1 was expressed and purified as described in Rogers et al. (Rogers, B.L. et al., International Symposium – Workshop, Molecular Biology and Immunology of Allergens (1992), CRC-Press, Inc.
15 (in Press)). TRFP chain 1 was approximately 96% pure and contains the amino acids glycine and serine from a thrombin cleavage recognition site and the N-terminus followed by the complete 70 amino acid TRFP chain 1 sequence (Ohman, J.L. et al., J.
20 Immunol. (1974) 113:1668-1677).

Example 1

Subcutaneous peptide inhibits the response to subsequent peptide challenge

To assess the ability of subcutaneously
25 administered peptide to affect a T cell response, two groups of five B6CBAF$_1$ mice (Age matched B6D2F$_1$ (H-2^{bxa}), Jackson Lbas, Bar Harbor, ME) 6-10 week old female mice) were treated with subcutaneous injections of either peptide Y in PBS or PBS alone.
30 B6CBAF$_1$ mice were chosen because of their strong T cell response to peptide Y. The mice were then challenged with peptide Y in CFA. A peptide specific T cell response was measured by peptide Y-specific

IL-2, IL-4, and IFN-γ production 10 days after
challenge. As shown in Table 1, peptide Y specific
production of all of the examined cytokines by lymph
node or spleen cells was decreased in cells from the
05 peptide-tolerized animals. Note that in each of the
cytokine assays there were similar low backgrounds
using cells from both groups of animals in cultures
with no peptide Y. This experiment demonstrates that
subcutaneous peptide injection can induce peptide
10 specific T cell tolerance as shown by the inability
of the cells to specifically produce cytokines. Note
that production of IL-4 was decreased in the cultures
from peptide-tolerized animals. In addition,
antigen-specific T cell proliferation was decreased
15 in the peptide-tolerized animals

-35-

Table 1.   Tolerization by s.c. injection with a peptide decreases the T cell response to that peptide.

Subcutaneous tolerization*	cells†	(μg/ml) In vitro IPC-2	(cpm x 10^{-3}) IL-2	(cpm x 10^{-3}) IL-4	(O.D. 450nm) IFN-γ
PBS	lymph node	150	49.86 (6.46)‡	2.82 (0.29)	1.20 (0.02)
		0	2.61 (0.98)	0.56 (0.03)	0.06 (0.01)
—	spleen	150	31.62 (4.54)	13.31 (2.96)	1.14 (0.01)
		0	2.72 (0.85)	1.17 (0.14)	0.06 (0.01)
IPC-2	lymph node	150	6.82 (0.64)	1.04 (0.08)	0.26 (0.11)
		0	2.60 (0.74)	0.39 (0.03)	0.08 (0.01)
	spleen	150	4.53 (0.32)	1.09 (0.20)	0.09 (0.01)
		0	1.60 (0.70)	1.09 (0.11)	0.07 (0.01)

* Five naive B6CBAF₁ mice were tolerized by subcutaneously administering either IPC-2 peptide in PBS or PBS alone. They were then challenged with IPC-2.

† Draining lymph nodes and spleens were removed and pooled and cells were cultured with IPC-2 as shown. Supernatants were analyzed for cytokine production.

‡ Each number represents the arithmetic mean of values from triplicate cultures plus and minus the SEM, shown in parentheses.

Example 2

Subcutaneous and intravenous administration of
peptide Y induces T cell tolerance in mice

Four groups of five BDF1 (C57BL/6J x DBA/2J)
05 mice (females, 6-8 weeks of age), were injected
either subcutaneously in a single dorsal site between
the forelimbs or intravenously through one of the
tail veins. For each injection site on day 0 and day
5, one group of animals was injected with 300 µg
10 peptide Y in Phosphate Buffered Saline (PBS) and the
other was injected with PBS alone. On day 10, each
animal was challenged with 100 µg peptide Y in 200 µl
Complete Freuds adjuvant (CFA) in four sites, two
subcutaneous sites at the base of the tail and two
15 subcutaneous sites on the thigh. The animals were
sacrificed by cervical dislocation on day 20 and
inguinal and popliteal nodes were removed and placed
in rinsing buffer, cold RPMI 1640 containing 1% Fetal
Bovine Serum (FBS). The nodes were rinsed with
20 rinsing buffer and forced through a fine stainless
steel mesh, using a glass pestal to suspend them in
rinsing buffer. The suspended cells were rinsed two
times by centrifugation at 1500 rpm for 10 minutes
and resuspended with rinsing buffer. An aliquot of
25 suspended cells from each animal was counted on a
Coulter Counter Model ZB.

The cells (4×10^6/ml) were incubated in culture
media (RPMI media containing 10% FBS, 2 mM
L-glutamine, 50 U/ml penicillin, 50 µg/ml
30 streptomycin, and 5×10^{-5} M 2-mercaptoethanol) and
with either 150 µg/ml peptide Y (Fig. 4) or alone.
The 0.2 ml cultures were done in triplicate in flat
bottom 96 well plates (Costar) at 37°C and 5% CO_2.
After 24 hours, 50 µl of the media from each well was

placed in separate round bottom 96 well plates
(Costar) and was frozen overnight at -20°C to
eliminate carryover of live cells. The supernatants
were tested after thawing for their ability to
05 support the growth of CTLL-2, an IL-2 dependent T
cell clone (ATCC TIB#214). CTLL-2 in log phase
growth were rinsed 2 times by centrifugation at 1000
rpm for 10 minutes, aspiration of the media, and
resuspension of the pellet with culture media. CTLL
10 were added to the warmed culture supernatants (5 x
10^3 CTLL cells/well) and the IL-2 assay was incubated
at 37°C and 5% CO_2. After 24 hours, 1 µCi/ml
^{3}H-thymidine was added in 50 µl/well and the CTLL
cells were incubated an additional 4-6 hours, then
15 they were harvested with a Tom-Tec 96-well cell
harvester. The ^{3}H-incorporation in each well was
counted by a Betaplate Model 1205 scintillation
counter. Background counts were not subtracted.

     Each bar on Figure 4 represents the arithmetic
20 mean of triplicate in vivo cultures from one mouse.
After in vivo challenge with peptide Y in CFA, lymph
node cells from mice which were administered saline
by either route specifically responded to challenge
with peptide Y in vitro, as shown by IL-2 production
25 (white bars). In contrast, draining lymph node cells
isolated from mice who were administered peptide Y
intravenously and subcutaneously exhibited a
decreased specific IL-2 secretion following challenge
with peptide Y in vivo. (dark bars). The results of
30 this experiment demonstrate that subcutaneous and
intravenous administration of peptide Y results in T
cell tolerization as shown by the decrease in antigen
specific production of IL-2.

Example 3

Subcutaneous administration of peptide Y dose
response for induction of T cell tolerance in mice

In another experiment, BDF$_1$ mice were tolerized
05 with various doses of peptide X and peptide Y prior
to challenge with the peptide in order to determine
the lowest dose necessary for the induction of
tolerance. Seven groups of mice, each group
containing three BDF$_1$ mice, were injected
10 subcutaneously with 0.2 ml PBS containing from 0 -
500 µg/ml peptide Y (Figure 5) or peptide X (Figure
6). Each group of animals was injected at a single
dorsal site under the skin between the forelimbs on
both day 0 and on day 5. On day 10 all of the
15 animals received a challenge injection of 100 µg
peptide Y or peptide X in 200 µl CFA as described
above. On day 20 the animals were sacrificed and
draining lymph nodes were removed as described
above. The lymph node cells were suspended, rinsed,
20 and cultured as above with or without 150 µg/ml of
peptide Y or peptide X, as appropriate. The
supernatants from the cultures were assayed for IL-2
as described above.

Each bar in Figures 5 and 6 represents the
25 average of triplicate cultures of pooled lymph node
cells from three mice. The baseline for control
cultures (i.e., cells cultured without peptide X or
peptide Y) for the IL-2 assay were approximately 1500
cpm (Figure 5) and approximately 300 cpm (Figure 6).
30 The dose of peptide per injection needed to
completely decrease peptide-specific IL-2 production
is between 4 and 20 µg/dose for these experiments
using two injections (day 0 and day 5). The results

demonstrate that BDF$_1$ strain mice can be tolerized at the T cell level with small doses of peptide X or peptide Y.

Example 4
05 Subcutaneous administration of a combination of peptide X and peptide Y induces T cell tolerance in mice

In order to determine whether peptide X and
— peptide Y can induce tolerance when injected in
10 combination, B6CBAF$_1$ mice (C57BL/6J x CBA/J) were tolerized and challenged with a mixture of the two peptides. B6CBAF$_1$ mice respond well to both peptide X and peptide Y, as measured by peptide-specific IL-2 production from lymph node cells isolated from mice
15 who were challenged with either peptide. In this experiment, one group of five B6CBAF$_1$ mice was injected with 300 μg/dose each of peptide X and of peptide Y. The animals were injected at a single dorsal site under the skin between the forelimbs on
20 days 0 and 5. On day 10 the animals received a challenge dose of 100 mg each peptide X and peptide Y in 270 μl total CFA in above location. On day 20 the animals were sacrificed and draining lymph nodes were removed as described above. The lymph node cells
25 were isolated, suspended, rinsed, and cultured as above with or without 150 μg/ml of peptide Y, peptide X, or a combination of peptide X and peptide Y. The supernatants from the cultures were assayed for IL-2 as described above.
30   Each bar in Figure 7 represents the arithmetic average of cpm values of triplicate cultures of pooled draining lymph nodes of five animals. Tolerization of animals with a combination of peptide

X and peptide Y results in T cell tolerance in these animals against both of the peptides, presented in vitro either separately or together.

Example 5

05 Peptide Y specific T cell response in mice preimmunized with TRFP is tolerized by peptide Y when administered subcutaneously

In order to demonstate that subcutaneous administration of peptide Y can induce T cell

10 tolerance in primed mice, mice were immunized with whole protein, TRFP, so that there would be T cell response to peptide Y derived from the TRFP protein. Peptide Y in the native protein structure is obscured by the protein conformation. Thus, following TRFP

15 administration, the mice do not produce antibody specific for peptide Y.

BDF$_1$ mice were each immunized with 100 µg TRFP in 200 µl IFA (Incomplete Freund's Adjuvant) in a single site intraperitoneally. After four months,

20 the mice were bled and grouped according to their anti-TRFP IgG antibody levels as follows. The mice were warmed with an infra-red lamp and bled through one of the tail veins by nicking the vein with a razor blade (Gilette Platinum Plus). The blood was

25 allowed to drip into a serum separating tube (Microtainer, Becton-Dickenson) and allowed to clot. The sera was then separated from the clot by centrifugation at 13,000 rpm. The sera was stored at -20° until use. The sera was assayed for

30 TRFP-specific IgG by ELISA. For the IgG assay, immunoaffinity purified TRFP was coated onto Nunc Polysorp 96-well plates by incubation of 50 µl/well of 2µg/ml TRFP in PBS. Unless mentioned, each

incubation step in this protocol was performed for 1
hour at 37°C. After rinsing with PBS, the wells were
incubated with 0.5% gelatin in PBS to block excess
binding to the plate. The plates were rinsed with
05 PBS containing 0.05% Tween 20. Sera were diluted
1/500 in PBS and incubated in triplicate on the
plates. After rinsing, the bound mouse antibody was
detected by incubation with biotinylated goat
anti-mouse IgG (Southern Biotechnology Associates)
10 diluted 1/10000 in PBS. The plates were rinsed and
streptavidin conjugated to horse radish peroxidase
(Southern Bio.Ass.) diluted 1/10000 was added for 20
minutes and incubated at room temperature. TMB
peroxidase substrate (Kirkegaard and Perry) was used
15 according to directions supplied. The resulting O.D.
values were read by an ELISA reader (Bio-Tek modal
#310) at 450 nm to quantitate the complexes bound to
TRFP protein on the plates. The mice were matched
for O.D. of the developed ELISA wells using the 1/500
20 dilution of each sera and separated into two matched
groups of three animals. The range of the values for
the TRFP specific IgG of 1/500 dilution sera from
these animals was between 1.7 and 2.2.

    To determine whether T cells from these TRFP
25 primed mice respond to peptide Y, three TRFP primed,
anti-TRFP antibody positive mice (not in the IgG
matched groups above, but with similar IgG levels)
were sacrificed by cervical dislocation and the
spleens were removed and placed in rinsing buffer.
30 Spleens were used because intraperitoneal injection
leads to strong T cell responses in the spleen. The
spleens were suspended and rinsed as detailed above
for lymph node cells. The cells were cultured as
above with 150 μg/ml peptide Y or with media alone.

After 24 hours the supernatants were removed and tested for the presence of IL-2 as previously described. The peptide Y specific IL-2 production shows that these mice responded to peptide Y 4 months after TRFP priming in IFA (Figure 8).

In addition, TRFP IgG matched groups of mice were tolerized with peptide Y. The tolerance of these animals to peptide Y was then shown by their failure to specifically secrete IL-2 _in_ _vitro_ following an _in_ _vivo_ challenge with peptide Y. The IgG matched groups were tolerized and challenged with peptide Y as follows. One of the two IgG matched groups was injected subcutaneously on days 0 and 5 (day 109 and day 114 after TRFP priming) with 0.2 ml PBS containing 300 µg peptide Y. The other group was injected with PBS only. Both groups were injected at a single dorsal site between the forelimbs. On day 10 (day 119 after TRFP priming) both groups of animals were challenged with 100 µg peptide Y as described above. On day 20 (day 129 after TRFP priming) the animals were sacrificed and the draining lymph nodes were removed as described above. The lymph nodes were suspended, rinsed, and cultured as described above with 150 µg/ml peptide Y (Figure 9, left panel) or with media alone. After 24 hours, the supernatants of the cultures were tested for IL-2 as described above.

Each bar in the left panel of Figure 9 represents the mean of triplicate cultures of one mouse. This experiment demonstrates that T cells specific for peptide Y can be tolerized in animals which have been preimmunized with TRFP and demonstate a peptide Y specific T cell response at the time of tolerization.

Example 6

sc tolerization with peptide Y decreases the peptide
specific antibody response in animals preimmunized
with TRFP

05   The ability of mice to mount an antibody
response to peptide Y can be used as an assay for
peptide Y specific T helper cell activity. As shown
in Example 5, immunization of BDF1 mice with TRFP
generates antibodies specific for whole TRFP, with no
10 detectable antibodies specific for peptide Y. The
two matched groups of mice described above were bled
at the time of sacrifice (ten days after peptide
Y/CFA challenge). The serum was separated as
previously described. The sera were assayed both for
15 IgG antibody binding specifically to peptide Y. For
the IgG assay, immunoaffinity purified peptide Y was
coated onto Nunc Polysorp 96-well plates by
incubation of 50µl/well of 20µg/ml peptide Y in PBS.
Each incubation step in this protocol lasts 1 hour at
20 37°. The wells were incubated with gelatin in PBS
for blocking excess binding to the plate. Sera were
diluted in PBS in a range from 1/300 to 1/8100 and
incubated in triplicate on the plates. After
rinsing, the bound mouse antibody was detected by
25 incubation with biotinylated goat anti-mouse IgG
(Southern Biotechnology Associates). Streptavidin
conjugated to horse radish peroxidase (Southern
Biotechnology Associates) was added to detect antigen
bound biotinylated antibody-complexes. TMB
30 peroxidase substrate (Kirkegaard and Perry) was used
according to directions supplied and the resulting
O.D. values were read by an ELISA reader (Bio-Tek
model #310) at 450 nm to quantitate the horse radish
peroxidase-containing complexes bound to peptide Y on

in any of these mice in this time frame. Thus, tolerization of the T cells specific for peptide Y had no effect on the anti-TRFP antibody in the 20 days following tolerization.

05   Table 2

ELISA analysis of specific IgG binding of mouse sera to TRFP

	ELISA O.D.-sera sampled before "tolerization" and challenge	ELISA O.D.-sera sampled after "tolerization and challenge
"tolerization" with PBS only		
– mouse #1	0.842	0.782
– mouse #2	0.580	0.503
– mouse #3	0.725	0.693
"tolerization" with peptide Y in PBS		
– mouse #1	0.670	0.492
– mouse #2	0.687	0.654
– mouse #3	0.686	0.585

The values given are averages of duplicate wells of 1/4500 dilutions of the sera.

Example 8
Subcutaneous administration of peptide Y induces
tolerance in IL-2 and IL-4 producing T cells in mice
primed with TRFP

05   There are several phenotypic classes of T helper
cells which can be differentiated by their production
of lymphokines. Th_1 cells produce IL-2 and
γ-interferon, as well as other lymphokines. Th_2
cells produce IL-4 and other lymphokines. IL-2
10 production is easily measured from primary in vitro
lymph node cultures through the IL-2 dependent CTLL-2
cell line. Similarly, IL-4 can be measured with
cultures of the IL-4 dependent CT4S cells (provided
by W. Paul). However, production of IL-4 from
15 primary in vitro culture is low and not
reproducible. In order to determine whether
tolerization with peptide Y effects both types of T
helper cells, we pooled lymph node cells from the two
groups of mice (described previously) that were
20 tolerized, challenged, and assayed for T cell
tolerance. The cells (2×10^7 total) were cultured
in 5 ml culture media containing 30 µg/ml peptide Y.
After 21 days incubation at 37°C and 5% CO_2, the
cells were rinsed two times with culture media and
25 replated at 4×10^6 cells/ml in triplicate 0.2ml
wells with or without 150 µg/ml peptide Y for both
the IL-2 production assay and the IL-4 production
assay. Samples (50 µl each) of the supernatants for
the IL-2 assay were removed at 24 hours and assayed
30 as detailed above. Samples for the IL-4 assay were
removed at 48 hours and stored at 4°C until use (2
days minimum). The IL-4 content was assayed by the
ability of the supernatants to support the growth of
the CT4S line. CT4S cells (1×10^4/well) were added

to the warmed culture supernatants and incubated 40
hours at 37°C and 5% CO_2. ^{3}H-thymidine (1 µCi/50 ml
culture media/well) was added for an additional 8
hours of culture to quantitate CT4S growth through
05 incorporation of the thymidine into DNA. The cells
were harvested after freezing the plates to dislodge
the slightly adherent CT4S cells. The plates were
harvested and counted identically to the IL-2 assays
detailed above.

10   For both the secondary IL-2 and the secondary
IL-4 assays (Figure 10), the average number of counts
from the triplicate 150 µg/ml peptide Y wells was
divided by the average number of counts from the
wells without peptide Y to determine a stimulation
15 index. This was necessary because the no antigen
background in the secondary cultures is more variable
than in primary cultures. The peptide tolerization
decreased the peptide specific production of both
IL-2 and IL-4 in these secondary in vitro cultures.
20 These data suggest that tolerization with peptide Y
decreases the antigen specific production of both
IL-2 and IL-4. The effect indicates a tolerogenic
effect by administration of peptide Y on both classes
of T helper cells.

25 Example 9
Subcutaneous administration of peptide X and peptide
Y decreases T cell response to recombinant chain 1 of
TRFP

        Four groups of B6CBAF$_1$ mice were "tolerized" by
30 subcutaneous injection with either PBS alone or PBS
containing recombinant chain 1 of TRFP; PBS
containing peptide X and peptide Y; or PBS containing
peptides Fel 3-1, peptide Y, peptide C, and peptide

D. The mice were injected with 150 µg/dose of either
the TRFP protein or each of the peptides (days 0 and
5). The PBS alone and the PBS/peptides were injected
in 300 µl/dose. The PBS/chain 1 of the TRFP protein
05 was injected in 100 µl/dose. All tolerizing doses
were at a single site under the dorsal skin between
the forelimbs. On day 10 all of the animals received
a challenge injection of 100 µg recombinant chain 1
of TRFP in 200 µl CFA as described above. On day 20
10 the animals were sacrificed and draining lymph nodes
were removed as described above. The lymph node
cells were suspended, rinsed, and cultured as above
with or without 150 µg/ml recombinant chain 1 of
TRFP. The lymph node cell cultures in this
15 experiment were each 0.1 ml. The supernatants from
the cultures were assayed for IL-2 as described above.

Each bar in Figure 11 represents the average of
IL-2 produced by triplicate cultures of lymph node
cells from five individual mice. IL-2 assays from
20 cultures of these lymph node cells without peptide
was approximately 500 cpm. The results show that
tolerization with peptide X and peptide Y and
tolerization with chain 1 of TRFP is effective in
decreasing IL-2 production specific for chain 1 of
25 TRFP after in vivo challenge with the chain 1 protein
TRFP. The results indicate that tolerization with
two peptides from chain 1 (peptide X and peptide Y)
are as effective as tolerization with four peptides
from chain 1 (Fel 3-1, peptide Y, peptide C and
30 peptide D) or as effective as tolerization with TRFP
chain 1 itself.

The objective of these studies was to analyze the extent of human IgE binding to peptide X and peptide Y derived from the TRFP chain 1 sequence. An ELISA assay had been previously performed to quantitate IgE binding to TRFP with cat allergic patient plasma as the source of IgE. This assay format was then extended to analyze the potential for anti-TRFP IgE to bind peptides X and Y. These assays also included equal mixtures of the two peptides and each peptide separately conjugated to HSA (human serum albumin). Dot blot assays on some of these antigens were also run as an alternative means of detecting IgE binding. The clinical implications would be that if these peptides bind a significant portion of anti-TRFP IgE, adverse allergic reactions would be more likely during treatment of an allergic individual.

ELISA

The basic format of these ELISA assays was a direct binding assay wherein the antigen was coated onto the wells of a 96 well microtitre dish and then antibodies in solution are added and assayed for the binding capacity. The protocol for these assays was as follows:

Corning assay plates (#25882-96) were coated with 10 µg/ml of each coating antigen; affinity purified TRFP, peptide X, or peptide Y, peptide X and peptide Y mixed, peptide X/HSA, or peptide Y/HSA in PBS (phosphate buffered saline), at 50 µg/well and incubated overnight at 4°C. The peptides were conjugated to HSA (human serum albumin) by two

different methods based on their chemical structure.
The conjugation reaction with peptide X used the
reagents and protocol of the Pierce Imject Immungen
EDC conjugation kit (Pierce, Rockland, IL). Peptide
05 Y was conjugated to HSA through its cysteine residue
using the sulfo-SMCC reagent (Pierce). Both
conjugates were filter purified and dialyzed against
PBS. In the ELISA assay, the unbound coating
antigens were removed and the wells were blocked with
10 0.5% gelatin in PBS, 200 µl/well for 2 hours at room
temperature. The antibody solution was a pool of
plasma samples from 20 patients that were skin test
positive for commercial cat extract which was
serially diluted with PBS-Tween 20 (PBS with 0.05%
15 nonionic detergent Tween-Sigma, St. Louis MO) and
100µl/well was added and incubated overnight at 4°C
(plasma dilutions are tested in duplicate). This
plasma pool had been depleted of most of the IgG
antibodies by extraction with Protein G-Agarose
20 (GammaBind G-Agarose, Genex Corp. Gaithersburg, MD).
The second antibody (biotinylated goat anti-Human
IgE, 1:1000 Kirkegaard & Perry Laboratories Inc.
Gaithersburg, MD), was added at 100µl/well for one
hour at room temperature. This solution was removed
25 and streptavidin-HRPO, 1:1000, (Southern
Biotechnology Associates, Inc., Birmingham, AL) was
then added at 100/µl/well for one hour at room
temperature (all wells were washed three times with
PBS-Tween between each incubation step). TMB
30 Membrane Peroxidase Substrate system (Kirkegaard &
Perry Laboratories) was freshly mixed, and added at
100µl/well. The color was allowed to develop for 2-5
minutes. The reaction was stopped by the addition of
100µl/well of 1 M phosphoric acid. Plates are read

on a Microplate EL 310 Autoreader (Biotek
Instruments, Winooski, VT) with a 450nm filter. The
absorbance levels of duplicate wells were averaged.
The graphed results (log of the dilution vs

05 absorbance) of the ELISA assays are shown in Fig. 12.
Figure 12 is the graphic representation of ELISA
assays for TRFP, peptide X and peptide Y IgE
binding. Because IgE antibody is the least abundant
antibody in human plasma the first dilution, 1:2 in

10 PBS, shows an absorbance reading of 0.65 for TRFP
binding. Although clearly positive for binding, this
is still a low initial absorbance reading. Positive
binding is defined as absorbance readings that are
greater than or equal to two times the background

15 level. Some individual patients have much higher
levels of anti-TRFP IgE which is reflected in the
higher absorbance readings (data not shown). This
pool of IgE depleted plasma represents an average of
all the different patients anti-TRFP IgE. This assay

20 can accurately detect the relative quantity of
anti-TRFP IgE reactive with the coated TRFP
antigens. The two peptide antigens, peptide X and
peptide Y, do not demonstrate any specific IgE
binding at any of the dilutions examined. The

25 negative control for these binding assays is the
level of binding to wells which have had no antigen
coated on them. For the positive control for the
presence of peptide coated on the wells was an ELISA
using anti-peptide antisera against either peptide X

30 or peptide Y was used.
Table 3 shows the percent positive binding as
summarized data from different numbers of cat
allergic patients. A smaller set of patients was
examined for IgE binding to the mix of peptides and

the peptides conjugated to HSA. Again the positive
control for the presence of peptides or peptide
conjugates was binding of anti-peptide antisera (now
shown).

05   <u>Table 3</u>

      _ Summary of Analysis of Direct IgE Recognition of
                    peptide X and peptide Y

      ELISA*

            Antigens tested: Positive Binding   N

10   TRFP   100%   12
            Peptide X   0%   12
            Peptide Y   0%   12
            Peptide X+IPC-2   0%   4
            Peptide X/HSA   0%   3
15   Peptide Y/HSA   0%   3

      Dot Blot

            Antigens tested: Positive Binding   N

            TRFP   100%   8
            Peptide X   0%   8
20   Peptide Y   0%   8

      IgE binding studies using a similar protocol as
described above were carried out using peptides Fel
8-1, Fel 31-1, Fel 31-2, Fel 31-4, and Fel 31-3.
None of these peptides showed IgE binding.

<u>Dot Blots</u>:

The dot blot assay is similar to ELISA in that is a direct binding assay with the antigen bound to a solid phase matrix. In the case of the dot blot

05 assay the matrix is nitrocellulose paper and results are based on autoradiography with ^{125}I-Streptavidin binding to a biotin labeled second antibody.

Dot blot protocol: Antigen; TRFP, peptide X and peptide Y, were coated on 0.1 μm nitrocellulose sheet

10 using 96-well dot blot manifold (Biorad, Richmond, CA) for 45 min. at room temperature in 100 μl sample volumes. TRFP was coated at 2,0.2 and 0.02 μg/dot and the peptides at 10, 1 and 0.1 μg/dot. Wells were then washed with 200 μl PBS. After equilibration of

15 blots with Tween solution, (Tris-HCL; pH 7.5 0.025M NaCl 0.17m Tween-20 0.5%) all sections were blocked with 1% nonfat dry milk, 1% fetal calf serum/Tween solution for 1-1.5 hour. Patient plasma was used at 10% (v/v) in 1% milt/Tween solution and preabsorbed

20 on blank strips of nitrocellulose for 1 hour. After preabsorption, the diluted patient plasma sample was incubated with the antigen bearing dot blot overnight at room temperature (RT). Nitrocellulose dot blot was incubated in biotinylated goat anti-human IgE

25 (1:2500) in Tween solution for 2 hours RT. After washing, the sections were incubated in 1 μCi ^{125}I-Streptavidin, 1 hour. Following removal of unbound label by extensive washing, the blots were exposed to film for 16 hours.

30 The summarized results of the dot blot assays are shown in Table 3. At all concentrations the peptide antigen dots were negative for specific IgE binding and with this patient set all were positive for TRFP binding.

Conclusion:

By using the direct binding ELISA method we have
clearly demonstrated IgE binding to affinity purified
TRFP. With this same assay we detect no specific
05 binding of cat allergic IgE to peptide X and peptide
Y. For further demonstration of lack of peptide
binding, a mixture of the two peptides as the coating
antigens was examined. This mixture was negative for
detectable, specific IgE binding as were the
10 individual peptides which has been conjugated to
human serum albumin. The rationale for including
these forms was to check that the lack of binding was
not just due to some conformation of the peptide as
it is directly bound to the plate. These results are
15 correlated with those from the dot blot assay wherein
a distinctly different matrix is used that includes a
different end-point readout (i.e., autoradiography
versus enzymatic color development). No direct IgE
binding to Fel 8-3 and Fel 30-4 was detected.

20 Example 11
Histamine Release Analysis Comparing TRFP to peptide
X and peptide Y

The objective of the histamine release analysis
was to directly assay the effects of peptides or TRFP
25 in an in vitro allergic response system. The release
of histamine through IgE recognition and IgE receptor
crosslinking on live cells directly assays the
allergic potential of a protein antigen. The aim of
these studies was to compare this allergenic
30 potential between the known allergen TRFP and peptide
X and peptide Y.

The histamine release assay used for these
studies is based on the detection of an acylated

antibody (Morei, A.M. and [...] Allergy Clin. Immunol. 82:646-654.) This assay was performed as two separate protocols: 1) the release

05 of histamine from basophils present in heparinized whole blood in the presence of different concentrations of antigens and 2) the actual assaying of histamine present in the supernates of the release reactions following cell removal by centrifugation.

10 The reagents for this histamine radioimmunoassay are supplied commercially as a kit by Amac Inc. (Westbrook, ME).

Whole blood was drawn from cat allergic patients using heparinized syringes or vacutainers. The

15 antigens, affinity purified TRFP, peptide X and peptide Y, were diluted to 2x concentration in PACM (PIPES buffer 25mM, NaCl 110mM, KCl 5.0mM, human serum albumin 0.003%, $CaCl_2$ 5mM, $MgCl_2$ 2mM, pH 7.3) buffer with 0.2 ml in each 1.5 ml polypropylene

20 tube. The same volume of blood, 0.25 mls, was added to each tube and the reactions were started by inversion. The buffer control consisted of whole blood with just buffer and no added antigen. The release reactions were then carried out at 37°C for

25 30 minutes. After this incubation the tubes were centrifuged at 1500 RPM for 3 minutes and the supernates were carefully removed. It was important to spin gently so that there would be no cell lysis giving misleading results. For the total histamine

30 release values a 100 μl blood sample was boiled for 3 minutes in a total volume of 1 ml with PACM buffer. At this point the supernates were either frozen at -20°C for later analysis or processed immediately.

For the RIA analysis, a 50 µl aliquot of the
release supernatant was diluted 1:4 with Histamine
release buffer (supplied with the kit from Amac
Inc.) Then 100 µl of each diluted supernatant was
05 added to the tubes containing the acylation reagent
(a lyophilized powder). Immediately after the
addition of each supernatant, 50 µl of acylation
buffer was added and the tube mixed by vortexing
until the reactants were completely dissolved. The
10 acylation reactions were allowed to go to completion
at R.T. for $\geq$30 minutes. A set of histamine
standards was supplied with the kit and these all
were processed by the acylation reaction (i.e., 100
µl of each standard and 50 µl of acylation buffer in
15 each acylation reaction tube.) Then 50 µl of each
acylation reaction was added to the bottom of a
monoclonal antibody coated 12x75 mM plastic tube.
This monoclonal antibody recognizes the acylated
histamine derivative as its epitope. Finally, 500
20 µls of ^{123}I labelled tracer was added to each tube on
ice and the binding reaction was carried out
overnight at 4°C($\geq$ 18 hrs). The binding reactions
were stopped by aspiration of the solutions from each
tube. Then they were counted on a gamma counter
25 (Cobra 5005, Beckman Inc.) for two minutes/tube.
Following the quantitation of ^{125}I label/tube
the standard curve is generated from the histamine
standard counts and plotted on semi-log paper.
Because this is a competition assay, with labelled
30 ^{125}I tracer competing with the standards or unknowns
for cell supernatants, the lower the recorded counts
the greater the amount of cold acylated histamine in
the binding assay. The data points were then
generated by reading off the values on the plotted

This type of analysis for histamine is a very sensitive, accurate method of measurement.

05   Figure 13 shows the summarized graphed results of eight individual cat allergic patients comparing histamine release following culture with various concentrations of affinity purified TRFP, peptide X and peptide Y. The graph shows the concentration of

10 the various antigens in nMoles versus the percent total histamine released for each patient. The graph depicts the average percent release for each concentration point and the standard error of the mean are shown with error bars. Although only one

15 reaction/patient is performed with PACM buffer alone it is represented as a line for comparison with antigen release levels.

The results are represented as percent total histamine released because of the great variability

20 between patients in overall histamine levels. The source of this variability relates to the number of basophils per unit blood from patient to patient and the variation in quantity of histamine per basophil. There are only six (5-fold) concentration points for

25 the TRFP peptide antigens emphasizing the higher end of the concentration curve. The TRFP concentrations are eight five-fold dilutions with three lower concentrations used for TRFP to get the full response range.

30   The histamine release profile shown in Figure 13 demonstrates that TRFP, at all but the lowest concentration, gives a clear release signal. However, there is the appearance of a plateau of release at lower concentrations with increasing

release at higher antigen concentrations. The
typical histamine release profile with a single,
purified allergen should theoretically show a
bell-shaped curve with lower histamine released at

05 higher concentrations due to lack of crosslinking
(i.e., each IgE molecule bound to a cell surface
receptor binds a separate allergen molecule). This
was not the case in the current experiments with
TRFP. There is no discernible histamine release to

10 either peptide X or peptide Y.

Example 12
Reassociation of TRFP Recombinant Chains 1 + 2
    The two recombinant chains of TRFP were
reassociated in vitro as a means of increasing IgE

15 reactivity and generate a reagent that more closely
resembles the native Fel d I which can be used in its
place, especially for those assays that require a
large quantity of material. The reassociated TRFP
may be useful as a diagnostic reagent.

20   The protocol used for the reassociation required
1 mg of each recombinant chain (rchain) mixed under
the following conditions;

Reaction conditions
1) 1 mg of rchain 1&2, 2) 6.56M Urea (final conc.)

25 3) 50 mM DTT 4) 0.2X phosphate buffered saline (PBS)

    The final volume was 5 mLs and was heated to
55°C for 3 minutes with shaking and then 5 mLs H_2O
was added and the mix was allowed to cool to room
temp. This was followed by extensive dialysis

30 against 1X PBS at room temp using a membrane with a
molecular weight cutoff of 3,500.

The reassociated TRFP was analyzed by ELISA followed by a depletion ELISA. The protocol for the ELISA is as follows: Microtiter plates were coated with 5.0μg/mL of coating antigen (Fel d I, recombinant TRFP (rTRFP) chain 1, rTRFP chain 2, a mix of rTRFP chain 1+2, or reassociated rTRFP chain 1+2) in PBS at 100μL/well and incubated overnight at 4°C. The plates were washed three times between each step with PBS-T (Phosphate buffered saline + 0.05% Tween 20). The unbound antigen was removed and the plate blocked with 300μL/well of 1% bovine serum albumin (BSA) in PBS-T for one hour at room temperature. All subsequent reagents were added at 100μL/well. For direct ELISA, serially diluted human plasma was added to duplicate wells and incubated overnight at 4°C. This was followed by biotinylated goat anti-human IgE (1:1,000) for one hour at room temperature, then streptavidin-HRPO (1:10,000) for one hour at room temperature. TMB substrate and H_2O_2 were freshly mixed and added; the color was allowed to develop for 2-5 minutes. The reaction was stopped by the addition of 1M phosphoric acid. The plates were read on a Dynatech plate reader at 450nm and the absorbances of duplicate wells were averaged. For the depletion ELISA, patient plasma was pre-incubated on antigen or PBS coated wells, plasma collected and re-incubated on freshly coated wells. The ELISA was then performed as outlined above.

The results of the direct ELISA shown in Fig. 21 demonstrated that the reassociated TRFP had similar binding properties to native Fel d I, the two separate recombinant TRFP chain and the mixture of rchain 1 and rchain 2. As shown in Fig. 22, the depletion ELISA demonstrated that the reassociated

material had properties more closely matching Fel d I than either the two chains separately or mixed. Pre-absorption on Fel d I showed the greatest depletion of human IgE against Fel d I (lowest binding curve); pre-absorption on the reassociated chains exhibited significantly greater reduction of Fel d I specific IgE (second lowest binding curve) than pre-absorption on a mix of the chains or the individual chain. It appeared that the reassociated recombinant chains either have more epitopes for IgE binding (than a mixture of rchain 1 and rchain 2) and/or that the affinity of binding to the epitopes has been increased.

EXEMPLIFICATION B

In another set of experiments, following a different methodology, T-cell proliferation, IL-2 production and IL-4 production for four cat allergic patients was studied. These experiments examined the ability of peptides comprising epitopes of TRFP to induce in vitro proliferation of T cells from cat allergic patients and whether this proliferation can be linked to the synthesis of the cytokines, interleukin 2 (IL-2) and interleukin 4 (IL-4). In addition the peptides shown in Tables 4 and 5, peptides Fel-32, Fel-33, Fel-34, Fel-35, Fel-36, Fel-37, Fel-38, Fel-38-1, Fel-39, and Fel-39-1 as shown in Figure 14 were included in these experiments.

Table 5

Response of Cat Allergic Patients to Chain 2
TRFP peptide

Peptide	Amino acid	PI	SI	N
Fel 16	1 − 22	283	5.9	52
Fel 17	12 − 33	421	6.1	114
Fel 32-1	12 − 24	442	6.6	21
32-2	14 − 24	424	5.3	20
32-3	16 − 24	270	3.7	22
Fel 18	23 − 48	466	6.3	99
Fel 33-1	26 − 36	340	5.4	63
33-2	26 − 38	210	4.2	50
33-3	26 − 40	235	5.0	47
Fel 31-1	14 − 40	733	9.4	36
31-2	14 − 39	599	8.1	35
31-3	14 − 38	598	8.3	36
31-4	14 − 37	622	8.4	35
31-5	14 − 36	539	7.6	37
31-6	15 − 40	295	4.4	33
31-7	15 − 36	267	5.8	33
Fel 20-1	34 − 59	395	5.9	79
Fel 25	49 − 68	350	7.6	56
Fel 28	60 − 82	94	3.6	43
Fel 28-1[1]	60 − 82	176	5.5	44
Fel 29	74 − 92	259	5.5	47

1   Based on short form chain 2 sequence (C2S)

Human T cell lines from four cat allergic
patients (patients 688, 730, 738, and 807) were
isolated. Heparinized peripheral blood specimens
were obtained from cat allergic patients and the
05 mononuclear cell fraction was purified by
Ficoll-Hypaque centrifugation. An aliquot of these

cells (2×10^6) was stimulated in vitro with purified
native TRFP (10 μg/ml) and grown in the presence of
recombinant IL-2 and IL-4. The T cell line was then
rested in culture until ready for secondary
05 proliferation assay.

    Two x 10^4 cells were cultured in 200 μl medium
containing various concentrations of test antigens
with 5×10^4 γ-irradiated autologous Epstein-Barr
Virus transformed cells as antigen presenting cells.
10 After 3 days of culture, each microwell was pulsed
overnight with 1 μCurie tritiated thymidine, and the
amount of radioactivity incorporated was measured by
liquid scintillation counting. The stimulation index
(S.I.) for each antigen was then calculated. The
15 S.I. is defined as the maximal counts/min for each
antigenic stimulation divided by the medium control
counts/min.

    Two x 10^6 rested T cells were cultured in 1 ml
of medium containing 20 μg/ml of test antigesn with 2
20 x 10^6 γ-irradiated autologous Epstein-Barr Virus
transformed cells as antigen presenting cells.
Identical controls received no antigen. After a 20
hour incubation, cells were separated from medium by
centrifugation. The IL-2 in the medium was measured
25 by proliferation of the IL-2 dependent T cell line,
CTLL-3. Cultures of 1×10^4 CTLL-3 were exposed to
three dilutions (25%, 5%, 1%) of sample supernatants,
incubated for 20 hours, and harvested after 4 hour
pulse with 1 μCurie tritiated thymidine. The IL-2
30 bioassay is sensitive to 2 pg/ml IL-2. IL-4 was
measured by an ELISA purchased from R&D Systems,
Minneapolis, MN. The IL-4 ELISA is sensitive to 16
pg/ml IL-4. The stimulation indices for IL-2 and
IL-4 production were calculated by dividing the

amount of IL-2 or IL-4 contained in the medium from stimulated T cells divided by the amount of IL-2 or IL04 released by non-stimulated T cells.

The results of these experiments shown in Figures 15-20. The results show that T-cells from cat allergic individuals respond to various peptides derived from the TRFP molecule. The stimulation index (S.I.) profile for T cell proliferation is similar to both the IL-2 production profile, and the IL-4 production profile for each of the patients (i.e. the same peptides shown to induce T cell proliferation were found to induce IL-2 and IL-4 production.) These results show that T-cell proliferation, IL-2 and IL-4 production are suitable methods for defining peptides which have T cell stimulating activity.

Futhermore, none of the epitopes stimulate IL-4 to the exclusion of IL-2. There do not appear to be peptides comprising epitopes which stimulate only IL-4, which is the cytokine responsible for IgE synthesis and subsequent allergic symptoms. However, one peptide, Fel 4, induces more IL-4 than IL-2 in this panel of four patients. The data shown in Figures 15-20 also show that interleukin production favors IL-4 as opposed to IL-2 when T cell lines from cat allergic individuals were cultured by TRFP.

EQUIVALENTS

Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific embodiments described herein. Such equivalents are considered to be within the scope of this invention and are encompassed by the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

   (i) APPLICANT: Gefter, Malcolm L.
                Garman, Richard D.
                Greenstein, Julia L.
                Kuo, Mei-chang
                Briner, Thomas J.
                Morville, Malcolm

   (ii) TITLE OF INVENTION: PEPTIDES USEFUL FOR TOLERIZATION

  (iii) NUMBER OF SEQUENCES: 14

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   (v) COMPUTER READABLE FORM:
      (A) MEDIUM TYPE: Floppy disk
      (B) COMPUTER: IBM PC compatible
      (C) OPERATING SYSTEM: PC-DOS/MS-DOS
      (D) SOFTWARE: ASCII TEXT

   (vi) CURRENT APPLICATION DATA:
      (A) APPLICATION NUMBER:
      (B) FILING DATE:
      (C) CLASSIFICATION:

  (vii) PRIOR APPLICATION DATA:
      (A) APPLICATION NUMBER: US 08/006,116
      (B) FILING DATE: 15-JAN-1993

      (A) APPLICATION NUMBER: US 07/884,718
      (B) FILING DATE: 15-MAY-1992

      (A) APPLICATION NUMBER: 07/857,311
      (B) FILING DATE: 25-MAR-1992

(viii)
    (A) NAME: Mandragouras, Amy E.
    (B) REGISTRATION NUMBER: 36,207
    (C) REFERENCE/DOCKET NUMBER: IPC-031

  (ix) TELECOMMUNICATION INFORMATION:
    (A) TELEPHONE: (617) 227-7400

(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 422 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (ix) FEATURE:
      (A) NAME/KEY: CDS
      (B) LOCATION: 8..286

    (ix) FEATURE:
      (A) NAME/KEY: mat_peptide
      (B) LOCATION: 74..286

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
CTGCATC ATG AAG GGG GCT CGT GTT CTC GTG CTT CTC TGG GCT GCC TTG        49
        Met Lys Gly Ala Arg Val Leu Val Leu Leu Trp Ala Ala Leu
        -22     -20             -15             -10


CTC TTG ATC TGG GGT GGA AAT TGT GAA ATT TGC CCA GCC GTG AAG AGG        97
Leu Leu Ile Trp Gly Gly Asn Cys Glu Ile Cys Pro Ala Val Lys Arg
            -5                  1               5


GAT GTT GAC CTA TTC CTG ACG GGA ACC CCC GAC GAA TAT GTT GAG CAA        145
Asp Val Asp Leu Phe Leu Thr Gly Thr Pro Asp Glu Tyr Val Glu Gln
 10                  15                  20


GTG GCA CAA TAC AAA GCA CTA CCT GTA GTA TTG GAA AAT GCC AGA ATA        193
Val Ala Gln Tyr Lys Ala Leu Pro Val Val Leu Glu Asn Ala Arg Ile
 25                  30                  35                  40


CTG AAG AAC TGC GTT GAT GCA AAA ATG ACA GAA GAG GAT AAG GAG AAT        241
Leu Lys Asn Cys Val Asp Ala Lys Met Thr Glu Glu Asp Lys Glu Asn
                 45                  50                  55
```

```
GCT CTC AGC TTG CTG GAC AAA ATA TAC ACA AGT CCT CTG TGT TAAAGGAGCC     293
Ala Leu S r Leu Leu Asp Lys Ile Tyr Thr S r Pro Leu Cys
        60                  65              70

ATCACTGCCA GGAGCCCTAA GGAAGCCACT GAACTGATCA CTAAGTAGTC TCAGCAGCCT     353

GCCATGTCCA GGTGTCTTAC TAGAGGATTC CAGCAATAAA AGCCTTGCAA TTCAAACAAA     413

AAAAAAAAA                                                              422
```

(2) INFORMATION FOR SEQ ID NO:2:

       (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 92 amino acids
          (B) TYPE: amino acid
          (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: protein

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Lys Gly Ala Arg Val Leu Val Leu Leu Trp Ala Ala Leu Leu Leu
-22     -20             -15             -10

Ile Trp Gly Gly Asn Cys Glu Ile Cys Pro Ala Val Lys Arg Asp Val
    -5                  1           5                       10

Asp Leu Phe Leu Thr Gly Thr Pro Asp Glu Tyr Val Glu Gln Val Ala
            15              20              25

Gln Tyr Lys Ala Leu Pro Val Val Leu Glu Asn Ala Arg Ile Leu Lys
        30              35              40

Asn Cys Val Asp Ala Lys Met Thr Glu Glu Asp Lys Glu Asn Ala Leu
    45              50              55

Ser Leu Leu Asp Lys Ile Tyr Thr Ser Pro Leu Cys
    60              65              70
```

(2) INFORMATION FOR SEQ ID NO:3:

       (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 428 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: cDNA

```
       (ix) FEATURE:
            (A) NAME/KEY: mat_peptide
            (B) LOCATION: 80..292

       (ix) FEATURE:
            (A) NAME/KEY: CDS
            (B) LOCATION: 26..292


     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGCCTGGCGG TGCTCCTGGA AAAGG ATG TTA GAC GCA GCC CTC CCA CCC TGC         52
                            Met Leu Asp Ala Ala Leu Pro Pro Cys
                            -18        -15                   -10


CCT ACT GTT GCG GCC ACA GCA GAT TGT GAA ATT TGC CCA GCC GTG AAG        100
Pro Thr Val Ala Ala Thr Ala Asp Cys Glu Ile Cys Pro Ala Val Lys
             -5                  1               5


AGG GAT GTT GAC CTA TTC CTG ACG GGA ACC CCC GAC GAA TAT GTT GAG        148
Arg Asp Val Asp Leu Phe Leu Thr Gly Thr Pro Asp Glu Tyr Val Glu
              10               15               20


CAA GTG GCA CAA TAC AAA GCA CTA CCT GTA GTA TTG GAA AAT GCC AGA        196
Gln Val Ala Gln Tyr Lys Ala Leu Pro Val Val Leu Glu Asn Ala Arg
         25               30               35


ATA CTG AAG AAC TGC GTT GAT GCA AAA ATG ACA GAA GAG GAT AAG GAG        244
Ile Leu Lys Asn Cys Val Asp Ala Lys Met Thr Glu Glu Asp Lys Glu
   40               45               50               55


AAT GCT CTC AGC TTG CTG GAC AAA ATA TAC ACA AGT CCT CTG TGT TAA        292
Asn Ala Leu Ser Leu Leu Asp Lys Ile Tyr Thr Ser Pro Leu Cys
              60               65               70


AGGAGCCATC ACTGCCAGGA GCCCTAAGGA AGCCACTGAA CTGATCACTA AGTAGTCTCA       352

GCAGCCTGCC ATGTCCAGGT GTCTTACTAG AGGATTCCAG CAATAAAAGC CTTGCAATTC       412

                                                                        428
AAACAAAAAA AAAAAA
```

(2) INFORMATION FOR SEQ ID NO:4:

       (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 88 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

       (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Leu Asp Ala Ala Leu Pro Pr  Cys Pro Thr Val Ala Ala Thr Ala
-18          -15              -10                      -5

Asp Cys Glu Ile Cys Pro Ala Val Lys Arg Asp Val Asp Leu Phe Leu
             1               5               10

Thr Gly Thr Pro Asp Glu Tyr Val Glu Gln Val Ala Gln Tyr Lys Ala
     15              20              25                      30

Leu Pro Val Val Leu Glu Asn Ala Arg Ile Leu Lys Asn Cys Val Asp
                 35              40                      45

Ala Lys Met Thr Glu Glu Asp Lys Glu Asn Ala Leu Ser Leu Leu Asp
         50              55                  60

Lys Ile Tyr Thr Ser Pro Leu Cys
         65                  70
```

(2) INFORMATION FOR SEQ ID NO:5:

   (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 485 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: cDNA

   (ix) FEATURE:
       (A) NAME/KEY: CDS
       (B) LOCATION: 8..337

   (ix) FEATURE:
       (A) NAME/KEY: mat_peptide
       (B) LOCATION: 59..337

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
TGACACG ATG AGG GGG GCA CTG CTT GTG CTG GCA TTG CTG GTG ACC CAA        49
        Met Arg Gly Ala Leu Leu Val Leu Ala Leu Leu Val Thr Gln
        -17     -15              -10                  -5

GCG CTG GGC GTC AAG ATG GCG GAA ACT TGC CCC ATT TTT TAT GAC GTC        97
Ala Leu Gly Val Lys Met Ala Glu Thr Cys Pro Ile Phe Tyr Asp Val
         1           5                  10
```

```
TTT TTT GCG GTG GCC AAT GGA AAT GAA TTA CTG TTG GAC TTG TCC CTC        145
Phe Phe Ala Val Ala Asn Gly Asn Glu Leu L u Leu Asp Leu Ser Leu
     15              20                  25

ACA AAA GTC AAT GCT ACT GAA CCA GAG AGA ACA GCC ATG AAA AAA ATC        193
Thr Lys Val Asn Ala Thr Glu Pro Glu Arg Thr Ala Met Lys Lys Ile
     30              35              40                  45

CAG GAT TGC TAC GTG GAG AAC GGA CTC ATA TCC AGG GTC TTG GAT GGA        241
Gln Asp Cys Tyr Val Glu Asn Gly Leu Ile Ser Arg Val Leu Asp Gly
                 50              55                  60

CTA GTC ATG ACA ACC ATC AGC TCC AGC AAA GAT TGC ATG GGT GAA GCA        289
Leu Val Met Thr Thr Ile Ser Ser Ser Lys Asp Cys Met Gly Glu Ala
                 65              70              75

GTT CAG AAC ACC GTA GAA GAT CTC AAG CTG AAC ACT TTG GGG AGA TGA        337
Val Gln Asn Thr Val Glu Asp Leu Lys Leu Asn Thr Leu Gly Arg
             80              85              90

ATTTTGCCAC TGATGCCCCT TCTGAGCCCC ATCCTCCTGC CCTGTTCTTT ACACCTAAAG      397

CTGGAATCCA GACACCTGTC CTCACCTAAT TCACTCTCAA TCAGGCTGAC TAGAATAAAA      457

                                                                        485
TAACTGCATC TTAAAAAAAA AAAAAAAA
```

(2) INFORMATION FOR SEQ ID NO:6:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 109 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Arg Gly Ala Leu Leu Val Leu Ala Leu Leu Val Thr Gln Ala Leu
-17     -15                 -10                  -5

Gly Val Lys Met Ala Glu Thr Cys Pro Ile Phe Tyr Asp Val Phe Phe
 1               5                  10                  15

Ala Val Ala Asn Gly Asn Glu Leu Leu Leu Asp Leu Ser Leu Thr Lys
                 20                  25                  30

Val Asn Ala Thr Glu Pro Glu Arg Thr Ala Met Lys Lys Ile Gln Asp
                 35              40                  45
```

```
Cys Tyr Val Glu Asn Gly Leu Il  Ser Arg Val Leu Asp Gly Leu Val
        50                  55                  60

Met Thr Thr Ile Ser Ser Ser Lys Asp Cys Met Gly Glu Ala Val Gln
        65                  70                  75

Asn Thr Val Glu Asp Leu Lys Leu Asn Thr Leu Gly Arg
    80                  85                  90
```

(2) INFORMATION FOR SEQ ID NO:7:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 27 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (v) FRAGMENT TYPE: internal

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Lys Arg Asp Val Asp Leu Phe Leu Thr Gly Thr Pro Asp Glu Tyr Val
1               5               10              15

Glu Gln Val Ala Gln Tyr Lys Ala Leu Pro Val
            20              25
```

(2) INFORMATION FOR SEQ ID NO:8:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 27 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (v) FRAGMENT TYPE: internal

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Lys Ala Leu Pro Val Val Leu Glu Asn Ala Arg Ile Leu Lys Asn Cys
1               5                   10                  15

Val Asp Ala Lys Met Thr Glu Glu Asp Lys Glu
            20                  25
```

(2) INFORMATION FOR SEQ ID NO:9:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 26 amino acids
      (B) TYPE: amino acid
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (v) FRAGMENT TYPE: internal

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Phe Phe Ala Val Ala Asn Gly Asn Glu Leu Leu Leu Asp Leu Ser Leu
1   5   10   15

Thr Lys Val Asn Ala Thr Glu Pro Glu Arg
      20   25

(2) INFORMATION FOR SEQ ID NO:10:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 19 amino acids
      (B) TYPE: amino acid
      (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (v) FRAGMENT TYPE: internal

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Glu Glu Asp Lys Glu Asn Ala Leu Ser Leu Leu Asp Lys Ile Tyr Thr
1   5   10   15

Ser Pro Leu

(2) INFORMATION FOR SEQ ID NO:11:

   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 19 amino acids
      (B) TYPE: amino acid
      (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Gly Glu Ala Val Gln Asn Thr Val Glu Asp Leu Lys Leu Asn Thr
1   5   10   15
Leu Gly Arg

(2) INFORMATION FOR SEQ ID NO:12:

   (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 16 amino acids
     (B) TYPE: amino acid
     (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: peptide

  (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Glu Glu Asp Lys Glu Asn Ala Leu Ser Leu Leu Asp Lys Ile Tyr Thr
1   5   10   15

(2) INFORMATION FOR SEQ ID NO:13:

   (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 14 amino acids
     (B) TYPE: amino acid
     (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: peptide

  (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Asn Ala Leu Ser Leu Leu Asp Lys Ile Tyr Thr Ser Pro Leu
1   5   10

(2) INFORMATION FOR SEQ ID NO:14:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 20 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (v) FRAGMENT TYPE: internal

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Thr Glu Glu Asp Lys Glu Asn Ala Leu Ser Leu Leu Asp Lys Ile Tyr
1   5   10   15

Thr Ser Pro Leu
       20

Claims

1. A composition comprising at least one peptide derived from a human T cell reactive feline protein, said peptide selected from the group consisting of peptide X (SEQ ID NO: 7), peptide Y (SEQ ID NO: 8), peptide Z (SEQ ID NO: 9), peptide A (SEQ ID NO: 10), peptide B (SEQ ID NO: 11), peptide C (SEQ ID NO: 12), peptide D (SEQ ID NO: 13), and peptide E (SEQ ID NO: 14), each as shown in Figure 3.

2. The composition of claim 1 wherein the at least one peptide is selected from the group consisting of peptide X (SEQ ID NO: 7), peptide Y (SEQ ID NO: 8), peptide Z (SEQ ID NO: 9), peptide A (SEQ ID NO: 10), peptide B (SEQ ID NO: 11) and peptide E (SEQ ID NO: 14).

3. A composition of claim 2, wherein the composition comprises peptide X (SEQ ID NO: 7).

4. A composition of claim 2, wherein the composition comprises peptide Y (SEQ ID NO: 8).

5. A composition of claim 1, wherein said composition comprises at least two peptides selected from the group consisting of peptide X (SEQ ID NO: 7), peptide Y (SEQ ID NO: 8), peptide Z (SEQ ID NO: 9), peptide A (SEQ ID NO: 10), peptide B (SEQ ID NO: 11) and peptide E (SEQ ID NO: 14).

6. A composition of claim 5, wherein the
   composition comprises peptide X (SEQ ID NO: 7)
   and peptide Y (SEQ ID NO: 8).

7. A therapeutic composition comprising a
   pharmaceutically acceptable carrier or diluent
   and at least one peptide derived from a human T
   cell reactive feline protein, said peptide
   selected from the group consisting of peptide X,
   peptide Y (SEQ ID NO: 8), peptide Z (SEQ ID NO:
   9), peptide A (SEQ ID NO: 10), peptide B (SEQ ID
   NO: 11), peptide C (SEQ ID NO: 12), peptide D
   (SEQ ID NO: 13) and peptide E (SEQ ID NO: 14),
   each as shown in Figure 3.

8. A therapeutic composition of claim 7 wherein
   said at least one peptide is selected from the
   group consisting of peptide X (SEQ ID NO: 7),
   peptide Y (SEQ ID NO: 8), peptide Z (SEQ ID NO:
   9), peptide A (SEQ ID NO: 10), peptide B (SEQ ID
   NO: 11) and peptide E (SEQ ID NO: 14).

9. A therapeutic composition of claim 8 wherein the
   composition comprises peptide X (SEQ ID NO: 7).

10. A therapeutic composition of claim 8 wherein the
    composition comprises peptide Y (SEQ ID NO: 8).

11. A therapeutic composition of claim 8 wherein
    said composition comprises at least two peptides
    selected from the group consisting of peptide X
    (SEQ ID NO: 7), peptide Y (SEQ ID NO: 8),
    peptide Z (SEQ ID NO: 9), peptide A (SEQ ID NO:
    10), peptide B (SEQ ID NO: 11) and peptide E
    (SEQ ID NO: 14).

12. A therapeutic composition of claim 11 wherein
     the composition comprises peptide X (SEQ ID NO:
     7) and peptide Y (SEQ ID NO: 8).

13. A method of treating sensitivity to _Felis_
     domesticus in an individual comprising
     administering to the individual a
     therapeutically effective amount of the
     composition of claim 7.

14. A method of claim 13 wherein the therapeutic
     composition is administered subcutaneously.

15. A method of claim 13 wherein the therapeutic
     composition is administered in soluble form.

16. A method of treating sensitivity to _Felis_
     domesticus in an individual comprising
     administering to the individual a
     therapeutically effective amount of the
     composition of claim 8.

17. A method of claim 16 wherein the therapeutic
     composition is administered subcutaneously.

18. A method of claim 16 wherein the therapeutic
     composition is administered in soluble form.

19. A method of treating sensitivity to _Felis_
     domesticus in an individual comprising
     administering to the individual a
     therapeutically effective amount of the
     composition of claim 12.

20. A method of claim 19 wherein the therapeutic composition is administered subcutaneously.

21. A method of claim 19 wherein the therapeutic composition is administered in soluble form.

22. A method of treating sensitivity to _Felis domesticus_ in an individual comprising administering to the individual a therapeutically effective amount of at least two therapeutic compositions, each composition comprising at least one peptide selected from the group consisting of peptide X (SEQ ID NO: 7), peptide Y (SEQ ID NO: 8), peptide Z (SEQ ID NO: 9), peptide A (SEQ ID NO: 10), peptide B (SEQ ID NO: 11) and peptide E (SEQ ID NO: 14), each peptide shown in Figure 3, said compositions each comprising a pharmaceutically acceptable carrier or diluent.

23. A method of claim 22, wherein one of the therapeutic compositions comprises peptide X (SEQ ID NO: 7) and one of the therapeutic compositions comprises peptide Y (SEQ ID NO: 8).

24. A method off claim 22 wherein each composition is administered in soluble form.

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25. A therapeutic composition useful in treating a
    disease which involves an immune response to a
    protein antigen, said therapeutic composition
    comprising at least one peptide which comprises
    a sufficient percentage of the T cell epitopes
    of said protein antigen such that in a
    substantial percentage of a population of
    individuals sensitive to the protein antigen,
    the response of such individuals to the protein
    antigen is substantially diminished, with the
    proviso that the at least one peptide does not
    comprise the entire protein antigen.

26. The therapeutic composition of claim 25 wherein
    the response of the T cells of such individuals
    to said protein antigen is substantially
    diminished.

27. The therapeutic composition of claim 25 wherein
    the protein antigen is an allergen.

28. The therapeutic composition of claim 25 wherein
    said therapeutic composition comprises at least
    two compositions.

29. A method of treating a disease which involves an
    immune response to a protein antigen comprising
    administering subcutaneously to a mammal a
    therapeutic composition comprising a peptide
    derived from the protein antigen and a
    pharmaceutically acceptable carrier, in an
    amount effective to tolerize T cells of the
    mammal to the protein antigen, wherein the
    peptide comprises at least one T cell epitope of
    the protein antigen.

TGACACGATGAGGGGGCACTGCTTGTCGCTGGCAATTGCTGGTGGTGACCCAAGGGCTGGGC   58
M R G A L L V L A L L V T Q A L G
-17 -15   -10   -5

GTCAAGATGGCGGAAACTTGCCCCATTTTTATGACGTCTTTTATGCGGTGGCAATTGAATGAAATGAATTACTGTTGGACTTGTCCCTCACA   140
V K H A E T C P I F Y D V F F A V A N G N E L L D L S L T
-1 5   10   15   20   25   30

AAAGTCAATGCTACTGAACCAGAGAGAGACAGCCATGAAAAAAATCCAGGATTGCTACGTGGAGAACGGACTCATATCCAGGGTCTTGGAT   230
K V N A T E P E R T A N K K I Q D C Y V E N G L I S R V L D
35   40   45   50   55   60

GGACTAGTCATGACACCATCAGCTCCAGCAAAGATTGCATGGGTGAAGCAGTTCAGAACACCGTAGAAGATCTCAAGCTGACACTTTG   320
G L V H T T I S S K D C M G E A V Q N T V E D L K L R T L
65   70   75   80   85   90

GGGAGAATGAATTTTGCCACTGATGCCCCTTCTGAGCCCCATCCTCCTGCCCTGTTCTTTACCTAAGCTGGAATCCAGACACCTGTCC   410
G R -

TCACCTAATTCACTCTCAATCAGGCTGACTAGAATAAAATAACTGCATCTTAAAAAAAAAAAAAAAA   485

FIG. 2

Sequence

peptide
name

X   KRDVDLFLTGTPDEYVEQVAQYKALPV

Y   KALPVVLENARILKNCVDAKMTEEDKE

Z   FFAVANGNELLLDLSLTKVNATEPER

A   EEDKENALSLLDKIYTSPL

B   MGEAVQNTVEDLKLNTLGR

C   EEDKENALSLLDKIYT

D   NALSLLDKIYTSPL
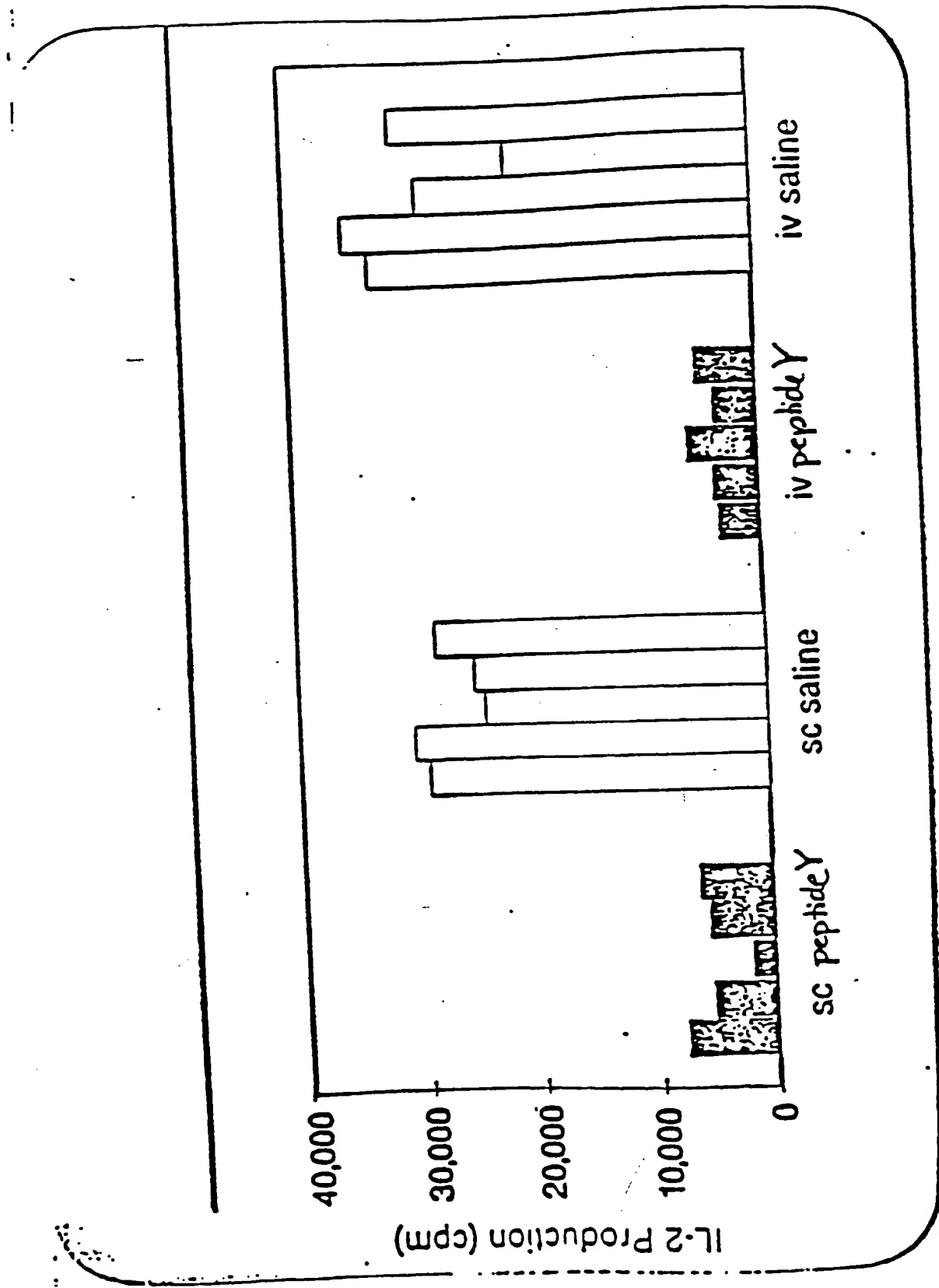
E   TEEDKENALSLLDKIYTSPL

FIG. 3

FIG. 4